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AGRA UNIVERSITY JOURNAL OF RESEARCH

Vol. I *(SCIENCE)* **November, 1952**

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AGRA UNIVERSITY JOURNAL OF RESEARCH

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Chief Editor.

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OURSELVES

VERY little apology is required for starting a JOURNAL of RESEARCH of the AGRA UNIVERSITY. The advancement of a University, indeed of a nation, depends on the research of its teachers and students. Research is really the main-spring of all knowledge and learning. The Agra University has just completed twenty-five years of its life. During this period, it has steadily grown and expanded and its affiliated colleges and institutions are scattered all over North and Central India. Its activities have not merely been confined to teaching and examining, but its alumni have also contributed to the advancement of knowledge in many branches. Schools of research and original investigation have flourished under its auspices and numerous original papers have been published in a great variety of scientific and other research periodicals in India and abroad. With the research workers of the University scattered as they are over a wide area, it is not easy for them to maintain contact with others. There has, therefore, been a great need for a JOURNAL OF RESEARCH. It seems appropriate that the Silver Jubilee of the University should be celebrated by starting such a journal. This journal will be published twice a year and issued separately in two parts, viz. *Science* and *Letters*. It will publish papers embodying the results of research work done in the University as well as critical reviews by specialists of recent researches. The response has been very encouraging and we have received a large number of original papers, some of them of great importance. In addition to messages of blessings and good wishes from many eminent men in the country for the new venture, we have also received ready co-operation and help of a large number of specialists, to whom these papers were referred for opinion. We offer them our heartfelt thanks. We are very grateful to Prof. Dr. M. S. Mani, Professor of Zoology & Entomology, School of Entomology, St. John's College, Agra for his willing co-operation and help in bringing out this issue. But for his ungrudging help in scrutinising the manuscripts, in preparing them for the press, in reading through the printer's proof sheets and in attending to a number of little details, the task would have been very difficult if not impossible. He has put in very hard work and we are deeply indebted to him. We are also grateful to Shri H. K. Kapur of the Agra University Press for the great pains he has taken in printing the Journal under very difficult conditions.

L. P. MATHUR
Registrar

Senate House,
Agra.
November 1, 1952.

FOREWORD

C. V. MAHAJAN
VICE-CHANCELLOR

A JOURNAL OF RESEARCH for the Agra University has been a long-felt need, since in an affiliating University like ours, with its colleges scattered over a vast area, it is not easy for research workers in one college to know what their colleagues in another college were doing. The first number of the Journal will appear at the time of the Silver Jubilee of the University, the subsequent numbers appearing twice annually. The Journal will contain papers which are the result of the writers' own advanced study or research and which have attained the requisite standard. The papers published in the first number show that in some of our colleges there are teachers capable of work of a high order, particularly in Science. It is my hope that a Journal like this will stimulate other teachers who have the talent but who have not yet conducted any research. Unless more of our teachers engage in advanced study and independent thinking, keeping abreast of modern developments and recent trends in their respective subjects, the academic standards of the University are bound to suffer, and good students in our colleges will fail to receive that guidance and inspiration which they should receive. The standards of any University must after all depend upon the standards of the individual teachers of that University.

AGRA UNIVERSITY
Agra, Sept. 12, 1952.

C. V. MAHAJAN
Vice-Chancellor

MESSAGES

GOVERNOR'S CAMP,
UTTAR PRADESH

August 30, 1952

THE AGRA UNIVERSITY, the largest university in Northern India, perhaps in the whole country, celebrates its Silver Jubilee in December, 1952. I deem myself fortunate that I happen to be its Chancellor on this occasion.

A University is not brick and mortar, teacher and students; it is not a degree conferring machine, nor a mechanism for holding examinations. It is, and should be, a powerful laboratory where all that is best in the past is studied, all that is best in the present is pursued, and all that is best for the future is made ready.

K. M. MUNSHI, CHANCELLOR

On this occasion, our UNIVERSITY and those who work for it must take a pledge to see that this aim is fulfilled in the near future.

On the professors lies the main responsibility, on the students is laid the primary duty, to redeem the pledge.

May they fulfil them both.

K. M. MUNSHI
Chancellor

VICE-PRESIDENT'S SECRETARIAT,
NEW DELHI,
22nd August, 1952.

My dear Vice-Chancellor,

IT is good to hear from you after such a long time.

I am glad to know that you are publishing a University *Journal of Research*. No person can be an effective teacher if he is not also an ardent seeker. Teaching and research should go together and the *greatness of a nation depends to a large extent on the research achievements of its workers*. I wish your Magazine every success.

Yours sincerely,
S. RADHAKRISHNAN

PROF. S. RADHAKRISHNAN
VICE-PRESIDENT
Republic of India.

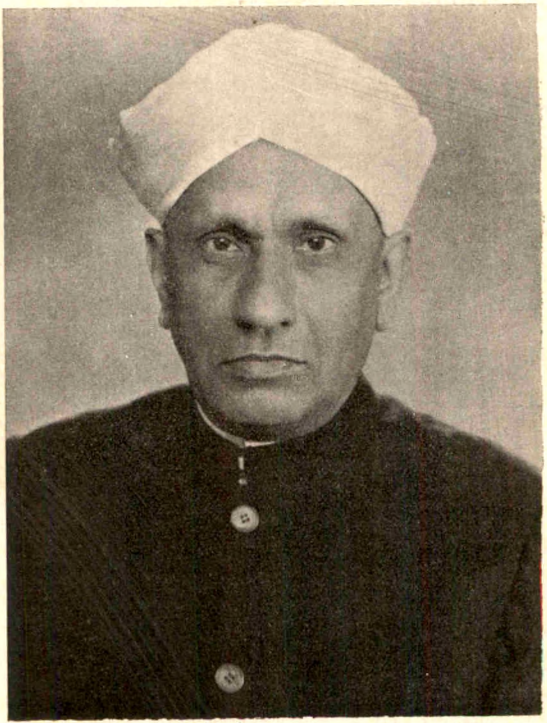
Sir C. V. RAMAN, F.R.S., N.L.,
Director.

RAMAN RESEARCH INSTITUTE
HEBBAL POST, *Bangalore 6*

23rd August 1952.

RESEARCH is the life-blood of University activities. The encouragement of research and the regular publication of its results are, therefore, *vital* necessities. It is entirely appropriate that in connection with the SILVER JUBILEE of the AGRA UNIVERSITY to be celebrated this year, it has been decided to bring out a University Journal regularly twice a year. It gives me much pleasure to convey my heartiest good wishes for the success of the Journal.

C. V. RAMAN



Yours sincerely
C. V. Raman
17. Sept 1952

IN the sixteenth century FRANCIS BACON could say, without immodesty, that he took all knowledge for his province. Those spacious days are gone, never to return. The bounds of knowledge have expanded and the most learned have to be content with a fringe of it. Specialization has become inevitable and one's labours and investigations have to be shared. One's excursions into undiscovered realms must engender a mood of humility and make one realize how little is known, whether of the world within us or outside of us. The main function of a teacher is, of course, the bringing up of youth, but a no less important duty is the advancement of learning and the addition to the sum of human knowledge. *The reputation of a University rests on good teachers as well as on good researchers and investigators.* The young UNIVERSITY OF AGRA has amply demonstrated that it has both. It is the hope of all its friends—and their number is large—that this *Research Journal* will contain the fruits of the labours of the scholars and teachers, will stimulate the younger generation in their pursuit of knowledge, and soon take its place among the more important journals of the country.

DR. AMARNATH JHA
CHAIRMAN
U.P. Public Service Commission

AGRA,
September 7, 1952.

AMARNATH JHA

Agra University
Journal of Research

Vol. I **Science** **November 1952**

CORRELATION OF THE GONDWANAS BASED ON THE EVIDENCE OF PLANT FOSSILS*

By SHIVDAYAL SAKSENA, *Darbar College, Rewa, India.*

ABSTRACT

All the previous works on the classification of the Gondwanas have been thoroughly studied and compared. Aiyengar's collection, on which Fox has based his views, has also been re-examined in original and an attempt has been made to acquire a first-hand knowledge of the localities of South Rewa Gondwana basin. Fresh collections have been made from some of these localities and examined. On the basis of the palaeobotanical evidence, a tripartite division of the Gondwanas—into Lower, Middle and Upper—has been suggested, and the stratigraphical sequence of the Ganjranalla, the Pali and the Parsora beds, has been clearly shown.

INTRODUCTION

The classification of the Gondwana system and the age of its various stages are subjects of great controversy. Geologists and palaeobotanists hold conflicting views. The fossiliferous localities in the South Rewa Gondwana basin gave a new start to the controversy and since then the problem became more and more intricate. In such cases the first-hand knowledge of the localities, and of their palaeobotanical and geological aspects proves of great help in the attempt of finding out a solution Moreover, in making fresh attempts at a solution both of the above aspects need be tackled together, as has already been done by the previous workers.

Vrendenburg, Feistmantel, Cotter, Fox and Sahni are amongst the foremost workers who expressed their opinion on the

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*Read at the *Symposium on the Stratigraphy of Gondwanaland* held under the presidentship of Dr. D. N. Wadia at the Fourth Scientific Meeting of the Palaeobotanical Society of India, at the Birbal Sahni Institute of Palaeobotany, Lucknow, on 10th & 11th January, 1952.

classification and the age of the Gondwanas, but no final agreement has been reached. Sahni (1926), on the basis of the fossil plant material and the data supplied to him by the Geological Survey of India, seemed more inclined to share Cotter's views. In the year 1932 he suggested to me to visit the South Rewa fossiliferous localities and make fresh collections. The present paper is based partly on my own collections and partly on Mr. N. K. N. Aiyengar's collection of 1929 (sent to Prof. Sahni by the Geological Survey of India) from several localities of the South Rewa Gondwana basin. I examined both of these collections under the able guidance of Prof. Sahni.

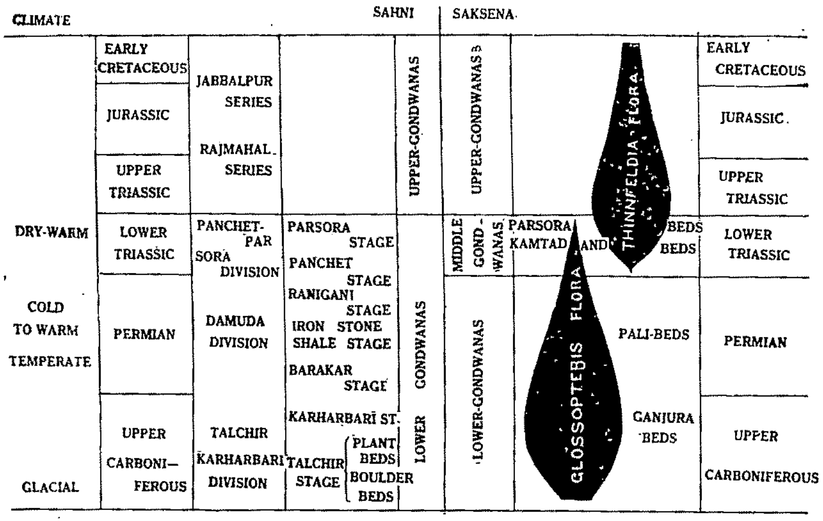
PREVIOUS WORKS AND THE PRESENT POSITION

E. Vrendenburg (Wadia 1926) and O. Feistmantel (1882) divided the Gondwanas into the Upper, Middle and Lower divisions, while G. de P. Cotter (1938) and C. S. Fox (1931) divided them into two—Upper and Lower—divisions only.

Vrendenburg included Maleri, Kamti and Panchet series in the Middle Gondwanas, while Feistmantel has included the Damudas also in addition to the above series. The creation of the Middle Gondwanas by Feistmantel has its own significance. He has taken two main characters—the lithology and the fossil contents —into consideration. Beds having similar lithological characters but differing in fossil contents have been included in this new division. He writes (Feistmantel 1882, p. 5): "There are certain red shales, from Aurangabad coal-field, which according to petrological conditions would be considered as belonging to Mahadevas (Upper Gondwanas), contain most decidedly Lower Gondwana fossils such as *Vertebraria, Glossopteris* and *Schizoneura.* There are certain beds in South Rewa showing the same thing. To provide for these doubtful cases and to comprise such beds on the boundary between the two former chief divisions with the Upper Gondwana aspect but with Lower Gondwana fossils the above subdivision (Middle Gondwana) is proposed." Feistmantel included the transitional beds (Parsora), Panchet division (Maleri, Kamti and Panchet) and the Damudas (Raniganj, Ironstone shales and the Barakars) in his newly created Middle Gondwanas;

and to this whole division he assigned the Triassic age. In Feistmantel's work the geological views are based on Hughes' notes (1881) who surveyed the South Rewa area and made his memorable collections with Hira Lal from over sixty localities.

Later on Sir Ceril S. Fox (1931, p. 189) after instituting a systematic search for fossils in the general area (Pali, Tiki, Umaria) nearly 500 sq. miles, which was ably carried out by Mr. N. K. N. Aiyengar of the Geological Survey of India, examined the area between Daigaon, Pali, Karkati and Parsora, which has been the subject of controversy for over 60 years and is the birth-place of the Middle Gondwana idea. He found that there is a steady northward dip of the beds from Pali (in south) to Parsora (7 miles north of Pali) and that the beds in the latter place must be at least 800 feet above those of Pali. He has satisfied himself that two distinct floras are present in the strata concerned—the Daigaon-Pali beds contain Damuda (Lower Gondwana) plant remains while the beds at Parsora have strong floral affinities with the Rajmahal (Upper Gondwana) plant remains. According to Fox the presence of *Cordites* (*Noeggerathiopsis*) in the Parsora beds has not been confirmed, while good specimens of *Neocalamites* have been discovered in these strata. Dr. Fox has satisfied himself that two



Shivdayal Saksena

separate rock series must be involved although the junction is masked, and that there is a strong floral break between them—the lower are the Pali-beds of the Kamti age and the upper is the true Parsora stage of the Rhaetic age. As regards the lithological similarity of Pali and Parsora beds, Fox is of the opinion that the colouration of the rocks is of the secondary nature and relatively superficial (Fox 1931, p. 189). He is almost definite that the red colour of the Supra-Barakars (Pali-Daigaon beds) of South Rewa was induced long after their original deposition (Fox 1934, p. 17).

As regards the sequence and the age of these beds, Fox (1934, p. 10) writes, "the Gondwana Era began with a glacial period in Upper Carboniferous times and closed at the beginning of the Cretaceous period..................this vast range of time can be divided into two chief periods—a lower (older) characterised by the so-called *Glossopteris* flora, and an upper characterised by a Cycadaceous *Ptilophyllum* flora..................The line of division between them is drawn about the middle of the Triassic period, when hot desert conditions appear to have prevailed in the Indian region." Fox is of strong view that the *Ptilophyllum* flora appeared after the total disappearance of the *Glossopteris* flora, and finds no stratigraphical reasons for recognizing a Middle Gondwana period at all.

Fox seems to place the Parsora stage, which has been the main cause of controversy, as high as possible in the Indian stratigraphical table, so much so that at places he has become inconsistent in his own views. At one place he regards Parsora stage (the term which he has restricted to the beds only around Parsora and not including the beds at Karkati and Daigaon as has been done by some other authors) as of the Rhaetic age—a little older than the Rajmahal series (Fox 1931, p. 82). In his opinion the Parsora beds contain no undoubted Lower Gondwana fossils and consequently he includes them with the Tiki beds (Mahadeva series) in the Upper Gondwanas (Fox 1934, p. 179). But at another place in the stratigraphical table recognized by the Geological Survey of India, he has shown Parsora stage with the Rajmahal series and has assigned it to the Lias of the Lower Jurassic age.

Cotter has divided Gondwanas into two divisions as has been done by Fox but disagrees from him in several details. He has also put the division line of the Upper and the Lower Gondwanas at the middle of the Triassic period, but the stratigraphical gap, which has been shown at the division line by Fox, has been indicated in the middle of the Jurassic period between Umia and Kota beds, and not in the middle of the Trias (Cotter 1938, p. 36). The Parsora stage has been assigned to the Lower Triassic age, and has been included in the Lower Gondwanas just above the Panchet stage, while Maleri beds have been classed with the Upper Gondwanas. It may be remembered that Cotter has included Daigaon, Karkati and Parsora beds, all the three under one stage—Parsora stage (Fox 1931, p. 189)—an the difference (which has been clearly shown in the comparative statement of the stratigraphical divisions of the Gondwanas given here) in the sequence of stages may be due to this reason.

Prof. Sahni (1921) has also used the terms Parsora stage and Parsora beds in the same broad sense as has been done by Cotter, and it is on this presumption that he says, "The small assemblage of plants found in the Parsora beds of India is thus full of interest. There is here a mixture of the past and the future: the past is represented by the typically Palaeozoic forms *Glossopteris*, *Vertebraria*, *Schizoneura*, *Cordaites*, *Samaropsis* and the peculiar *Squamae;* the future is heralded by *Cladophlebis denticulata*, a pre-eminently Jurassic species." It may be pointed out that all the above forms have not been found in the same bed and the different beds having groups of above fossil plants are situated actually at different horizons.

It seems to me that inclusion of the beds at Daigaon, Karkati and Parsora under the Parsora stage by Cotter and also by Sahni was based on Hughes' statement (1885) where he has put all the above beds under one wide heading of Supra-Barakars and which was taken as such by Feistmantel.

Prof. Sahni pointed out a marked palaeobotanical break above the Parsora stage. He felt that the Parsora flora contained strong Palaeozoic element. He agreed with Dr. Cotter's view that the Parsora beds should be included in the Lower Gondwanas (Sahni 1926). Prof. Sahni has suggested that the Parsora flora lived in the dry desert conditions of the Triassic period (Sahni 1935). He has assigned Lower Triassic age to the Parsora stage and has put it in the Panchet-Parsora division (Sahni 1937). He has further pointed out the mixture of Thinnfeldia flora and the Glossopteris flora at Parsora stage and also in the Panchet beds, which has also been mentioned by Fox (1931, p. 174) and described by Feistmantel (1880).

Aiyengar's collection of fossils from South Rewa localities was sent to Prof. Seward for identification and opinion; and probably he was given to understand that the whole of it was from Parsora beds. This is evident from his remarks on the flora (Seward 1933). Dr. Fermor who sent these fossils to Prof. Seward for identification was clear on this point. He writes (Fermor 1933), "It may be mentioned here that Lower Gondwana fossils like *Schizoneura* and *Glossopteris*, which occur in the Pali beds at Daigaon, also in Rewa, have not so far been found associated with the fossils of the above two localities (Parsora and Chicharia)."

A FRESH ATTEMPT

On the advice of Prof. Sahni, I made several collections from South Rewa localities, but the collection made in 1944 proved to be of great interest. This time I collected fossils from Ganjranalla, Daigaon, Karkati, Beli, Kamtadand and Parsora localities.

Ganjranalla beds situated near the junction of the nalla with the Johilla river (23·21 : 81·2) underlie the Barakar coal-seam (Hughes 1881, p. 126), which itself lies below the Barakar sand-stones. The fossil impressions are preserved in black carbonaceous shales. I have described the fossil flora of these beds under two heads—impressions and microflora. It consists of:—

Impressions—*Glossopteris indica* Schimper,
G. Browniana Feistmantel,
G. decipiens Feist.,

Gangamopteris cyclopteroides Feist.,
Noeggerathiopsis hislopi (Bunb.),
Samaropsis johillensis sp. nov.,
Samaropsis ganjrensis sp. nov.,
Some stem impressions.

Microflora — *Capsulites gondwanensis*, gen. et sp. nov.,

Some sporangium-like structures,
A variety of cuticles and tracheids,
A large number of microspores assigned to 19 genera and 66 species, and
Several megaspores belonging to one genus only.

Daigaon beds (23·22 : 80·59) overlie the Barakar sandstones and hence they have been placed by Hughes (1885, p. 72) under the heading of Supra-Barakars. The fossils found in the red ferruginous shales are preserved in very fragmentary state. The section starting from the coal-bed below the Barakar sandstones and going up to the red shales at the top is clear at several places in the Johilla river near these localities.

Fossiliferous beds near the forest rest-house at the ground level of a hillock at Karkati (23·22½ : 81·09) also consist of red ferruginous shales which have yielded a rich *Glossopteris* flora consisting of: —

Glossopteris indica Schimper,
G. Browniana Feistmantel,
G. angustifolia Feist.,
Sphenopteris polymorpha Feist.,
Dictyopteridium sporiferum Feist., and
an equisetaceous stem.

No microflora was found in these shales.

In going northwards from Karkati to Kamtadand and Parsora the northward dip of the strata can be very clearly seen at several places, and the view expressed by Fox (1931) that Parsora is higher in succession is perfectly justified. The fossils at Kamtadand and Parsora beds are also preserved in red ferruginous shales. At Kamtadand (23·25 : 81·08½) the fossil flora consists of :—

Glossopteris indica Schimper,
Vertebraria indica Royle,
Dicroidium hughesi (Feistmantel) and
an equisetaceous stem.

The Parsora beds (23·26 : 81·08) have yielded :—

Dicroidium hughesi (Feistmantel)
Vertebraria indica Royle and
Glossopteris sp. (?)

Aiyengar's collection has also been thoroughly studied jointly by Prof. Sahni and myself (Sahni and Saksena 1947). A part of this collection from the Ganjranalla locality was very kindly allowed by Prof. Sahni to be solely described by me. It yielded a new species of *Phyllotheca*, which subsequently has been named as *Phyllotheca sahni* by me (Saksena 1949).

DISCUSSION AND CONCLUSION

A brief survey of the work done in the South Rewa Gondwana basin helps us to arrive at certain definite and important conclusions. The tripartite division of the Gondwanas by Feistmantel was based on two different factors, (1) geological aspect of the fossiliferous rocks and (2) their fossil contents. He attached more importance to the lithological aspect, and irrespective of the nature of fossil contents, he included all the beds having similar lithological characters in the Middle Gondwanas. If we consider the palaeobotanical aspect of the beds included in the Middle Gondwanas we find that they fall into two distinct categories, one having purely Lower Gondwana fossils (*Glossopteris* flora) and the other having mixed elements of the *Glossopteris* and *Thinnfeldia* flora. The beds having the mixed flora are mentioned in his works as transitional beds, but he did not attach much importance to the palaeobotanical nature of these beds. This made the case of the Middle Gondwanas very weak. Taking advantage of this weak point, Fox shattered the Middle Gondwana theory to pieces. He ruled out the lithological criterion on the plea that the colouration of the Pali-beds is superficial and secondary. Now only the transitional beds of Parsora could support the Middle Gondwana theory on palaeobotanical grounds, but here too, by another strong assertion—that the Parsora beds have no undoubted Lower Gondwana fossils, and that there is no locality where the mixture of the Lower and the Upper Gondwana elements is found—Fox deleted the Middle Gondwana division and established the two partite division of the Gondwanas. Not only this he went further to suggest a floral break between the Lower and the Upper Gondwanas near about the Middle of the Triassic period. He firmly believed that the *Glossopteris* flora (Lower Gondwana flora) died out completely before the beginning of the *Thinnfeldia* flora (Upper Gondwana flora). To strengthen his view he separated the Parsora stage from Panchet series by placing the Mahadeva series in between *them.* It must be noted here that Panchets have greater floral affinities with Parsora than with the Mahadevas. Cotter, though in favour of two partite division, did not support this view. Sahni also did not feel satisfied with Fox's arguments. The floral break pointed out by Fox could not be proved beyond doubt. The mixed flora described by Feistmantel from Parsora could not be disproved, on the contrary the close examination of the specimen by Prof. Sahni and myself has established Feistmantel's view of the mixed flora at Parsora beds. Besides this I have also found the mixed flora (*Vertebraria* and *Thinnfeldia*) at Parsora as well as at Kamtadand. Thus these three instances of mixed flora conclusively disprove the floral break pointed out by Fox. They also help to bring Parsora beds next to Panchets, and prove the presence of the transitional beds at Parsora and Kamtadand on the basis of which the Middle Gondwana division can be re-established.

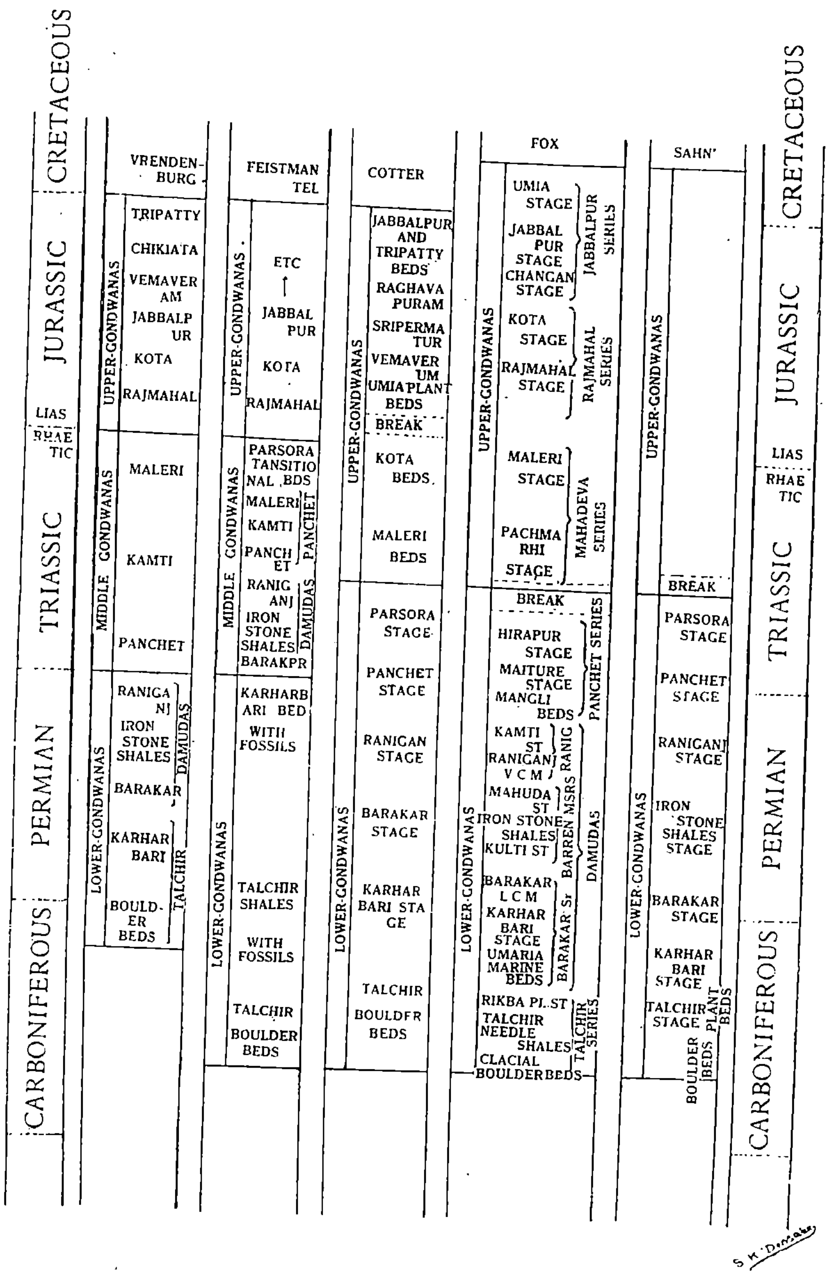
There is a complete change in the lithological as well as floral aspects between Parsora and Chicharia beds which supports the view of the floral break suggested by Prof. Sahni.

Hughes' inclusion of Daigaon, Karkati and Parsora beds under the heading Supra-Barakars created confusion. Cotter, Seward and Sahni formed an impression that the Daigaon, Karkati and Parsora beds belong to one and the same stage called Parsora stage. This led recently to a very serious mistake of including the Ganjranalla beds of Karharbari or Barakar age (Lower coal-measures) under the name of Pali-beds (Supra-Barakars of Hughes) by Virkki (1948) and Mehta (1945). Fox, with the help of Aiyengar, thoroughly studied South Rewa area and clearly showed that the Ganjranalla beds, Pali-beds of Karkati and Daigaon, and the Parsora beds belong to different strata, far apart from each other. Prof. Sahni also later on during the joint study made by us of Aiyengar's collection accepted the distinct stratigraphical position of the above beds.

Thus with the help of the fresh data collected by me and a thorough scrutiny of the previous works I have come to the following conclusions :—

> The Lower Gondwana flora (*Glossopteris* flora) appeared in the glacial period of the upper Carboniferous and flourished right up to the middle of the Triassic era. The vegetation was very rich during the latter part of the Upper Carboniferous period. Some genera attained maximum development in the Permian, while its decline began, producing weaker plants and lesser number of species in the beginning of the Trias and finally disappeared by the middle of that era. Its existence during the glacial and post-glacial times clearly shows that it was a cold and temperate climate flora, and its decline in the hot dry climate of the Triassic Era was natural.
>
> The Upper Gondwana flora (*Thinnfeldia* flora) made its first appearance in the Lower Triassic period (Panchet series) and rapidly attained prominence both in number of forms and development of structure. It reached its climax in the Upper Trias and the beginning of the Jurassic era, after which it gradually declined to extinction in the early cretaceous. Thus in the early half of the Triassic period both the Upper and the Lower Gondwana floras existed, the former was in its decline and the latter in its rise. It is this period which I am inclined to call, on the basis of purely palaeobotanical evidence as

Middle Gondwanas. The following chart would make the position clearer. Except the introduction of the Middle



Gondwanas it totally agrees with Prof. Sahni's views (1937). However I feel that on this basis alone the long disputed problem of the Middle Gondwanas cannot be finally settled. A regular systematic mapping of the whole area along with thorough palaeobotanical, lithological and stratigraphical study of the various localities concerned would be necessary before a final decision, agreeable to both, the palaeobotanists and the geologists, could be arrived at.

There is no floral break between Panchet and Parsora beds, but of course there is distinct floral change after the Parsora stage. The Parsora fossils have greater affinity with Panchet fossils than with Rajmahal fossils and so the Parsora stage is kept just after the Panchet series.

Ganjranalla beds, Pali-beds, Karkati and Daigaon and Parsora beds, in the South Rewa Gondwana basin, have distinct stratigraphical sequence which may be represented as below :—

Chicharia beds	(Upper Gondwana flora)
Parsora beds	(Mixed flora)
Kamtadand bed	(Mixed flora)
Pali beds (Supra-Barakars)	(Lower Gondwana flora)
Barakar sandstones	(Barren measures)
Barakar coal	(Lower coal-measures)
Ganjranalla beds	(Lower Gondwana flora)
Talcher boulder bed.	

ACKNOWLEDGMENTS

I owe a deep sense of gratitude to my teacher late Prof. Sahni who initiated me in this work and advised, guided and inspired me from the beginning till the end of my university career.

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LITERATURE CITED

Cotter, G. de P. 1938. The Indian Peninsula and Ceylon. *Regionale Geologie der Erde.* Band I, Leipzig.

Feistmantel, O. 1880. Fossil Flora of Gondwana system in India. *Palaeon. Indica,* series XII, **3** (2) : 51-56.

Feistmantel, O. 1882. Fossil Flora of Gondwana System of India. The Fossil Flora of the South Rewa Gondwana basin. *Mem. geol. Sur. India. Palaeon. Indica,* series XII, **4** (1).

Fermor, L.L. 1933. General report of Geological Survey of India. *Rec. geol. Surv. India,* **66** (ii) : 25.

Fox, C. S. 1931. The Gondwana System and the related Formations. *Mem. geol. Surv. India,* **58.**

Fox, C. S. 1934. The Lower Gondwana Coal-fields of India. *Mem. geol. Surv. India,* **59.**

Hughes, T.W.H. 1881. Notes on the South Rewa Gondwana basin. *Rec. geol. Surv. India,* **14.** 126-138.

Hughes, T.W.H. 1885. Southern Coal-fields of Rewa Gondwana basin. *Mem. geol. Surv. India,* **21.** (3) : 72.

Sahni, B. 1921. The present position of Indian Palaeobotany. *Proc. 8th Indian Sci. Cong. Calcutta. (Presidential Add. Bot. Sec.). Proc. Asiatic Soc. Beng. N.S.* **17** (4).

Sahni, B. 1926. The Southern Fossil Flora, a study of the plant geography of the past. *Presidential Add. Geol. Sec. 13th Indian Sci. Cong. Bombay* : p. 229-254.

Sahni, B. 1935. The Glossopteris Flora in India. *Proc. 6th Internat. Bot. Cong. Amsterdam,* **2** : 245-247.

Sahni, B. 1937. Speculations on the climates of the Lower Gondwanas of India, *17th Internat. Geol. Cong. Moscow* (abstract). p. 217-219.

Seward, A. C. 1933. Fossil plants from the Parsora stage, Rewa. *Rec. geol. Surv. India,* **66** (ii) : 235-243.

Virkki, C. (Mrs. C. Jacob,) 1946 Spores from the Lower Gondwanas of India and Australia. Reprinted from the *Proc. nat. Acad. Sci. India,* (1945). **15** (4 & 5) : 93-176.

Wadia, D.N. 1926. Geology of India for students pp. 116, 123-128.

FLORAL ANATOMY IN RELATION TO TAXONOMY*

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INTRODUCTION

The systematist is the indispensable referee to whom all workers, whether in morphology, cytology or physiology, have to refer their material for correct identification. His task, therefore, is a difficult one and unless we all help him by giving him new and reliable criteria for handling delicate problems, he will not be able to do his job well. It is true that in the past the work of floral anatomists, like that of wood anatomists, has been mistrusted or even ignored by the systematist. The fault, however, is mainly ours, for without realizing the limitation of our branch of study, we sometimes arrived at conclusions that from the point of view of a systematist were more or less improbable. On the other hand many taxonomists have been unduly sceptic about and indifferent towards contributions from anatomy. Thanks to the advice of such taxonomists as Camp (1940)† they now seem to be appreciating the value of cooperation with other branches of study. Let, therefore, the 'progeny' now realize that they have an obligation toward the 'sire' and if this is faithfully discharged, the 'sire' will be able to maintain itself in good stead to the benefit of all concerned !

THE SCOPE OF ANATOMICAL METHOD

It is a pity that so far the so-called anatomical studies of the flower are essentially confined to studying the course and distribution of vascular bundles within the receptacle and floral organs. Recent work has shown that epidermis and mesophyll characters in the study of foliage leaves have yielded useful taxonomic information. Floral anatomists would do well if they also make their studies more comprehensive than they have hitherto been.

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*Based on the matter prepared for a symposium on "Anatomy and Taxonomy" organized by the Botany Section of the Indian Science Congress, Bangalore, 1951.

†Referring to the indifference of 'morphology' and 'comparative anatomy' towards taxonomy, this Philadelphian taxonomist, (Camp 1940, p. 338), writes : "When they were born we (taxonomists) tolerated their blattings as we do those of infants. In adolescence we were blind to their needs and gave them little guidance and less help, refusing to see that as adults, they might have something of their own and something to contribute to our needs. It is therefore, little wonder that morphology, comparative anatomy and cytology pursuing their own ways with but little concern and less guidance from their parent (taxonomy !) should be lit. troubled with the trials and tribulations which now confront their sire."

The usefulness of the anatomical method is often believed to be due to the so-called conservative nature of vascular bundles. This point has recently been discussed at some length and the conclusion has been reached that the doctrine of conservatism of vascular bundles is unsound and is no longer tenable (Puri, 1951). While it is admitted that vascular bundles depend upon organs for their formation, it is also contended that once they are formed they attain some amount of autonomy with regard to their behaviour by virtue of the fact that they are not exposed to spatial difficulties to the same extent as are the organs which contain them. This, coupled with the fact that vascular bundles, so to say, do not ordinarily disregard morphological limits (*see* Puri, 1951) makes them fairly reliable in determining the past history of floral organs. It is in this way that they render valuable service to the systematist.

The limitation of this service, however, should always be borne in mind. Unlike genetical studies which help in determining relationships of smaller groups such as species, vascular anatomy of the flower usually helps in ranking groups of higher order such as genera and families. Sometimes, however, they may prove to be quite useful in specific identifications. For instance, the two common species of *Boerhaavia* can be easily distinguished from one another by the presence (*B. rependa*) or absence (*B. diffusa*) of marginal bundles in the perianth segments. Before we cite some more examples of how floral anatomy has helped to solve some taxonomic problems it will be worthwhile to discuss briefly the more important principles of floral variation which affect the vascular ground plan of the flower.

PRINCIPLES OF FLORAL VARIATIONS THAT AFFECT THE VASCULATURE OF THE FLOWER

In order to appreciate better the numerous modifications of the vasculature of flower it is desirable to assume some typical vascular plan as a starting point to which all the diverse patterns, if possible, can be referred as a standard. Such a plan may be assumed to occur in a typical pentacyclic flower, where sepals and carpels receive three traces each and petals and stamens (two whorls) only one trace each and where all these traces arise quite independently from the parent stele. Such a scheme, though purely hypothetical, serves as a good standard for measuring the extent of variations. During specialization and evolution of the flower this plan, like the external form, has undergone considerable modifications mainly through (1) Reduction, (2) Amplification, (3) Cohesion and (4) Adnation. These four principles, therefore, are of as much importance to the systematist as to the floral anatomist.

1. *Reduction.* Reduction in the number of vascular bundles in the flower is brought about through definite loss either of organs or of bundles in an organ or through fusion. In some cases entire whorls of organs particularly those of petals and stamens may be lost completely. In such cases the corresponding vascular bundles may also disappear completely but occasionally their '*trace*' parts—those parts which traverse the receptacular cortex as against the 'bundle' parts that traverse the organs—persist and give, as it were, more or less clear indications of the existence of such whorls in the ancestral forms. In the Polemoniaceae, for instance, there is typically a single whorl of stamens but anatomical evidence in the form of vascular stubs for a second whorl of stamens, which is now completely lost externally, is also available in some species (Dawson, 1936). Similar vascular stubs for lost whorls have also been reported in the Cyperaceae (Blaser, 1941), Lauraceae (Kasapligil, 1951), Caricaceae (Shanti Devi, 1952), etc.

Sometimes individual members of existing whorls disappear more are less completely. Here also their corresponding bundles may disappear altogether or their 'trace' parts may persist in the receptacular cortex as in *Leitneria* (Abbe & Earle, 1940), *Cercidiphyllum* (Swamy & Bailey, 1949), etc.

Occasionally the number of traces in an organ may be reduced. This is perhaps governed by physiological necessities although histological reason has also been advanced by some (see Puri, 1951). Among the dicotyledons sepals having only one trace each, instead of the usual three, have been reported for instance in *Menispermum* (Smith, 1928), *Calpoon* (Smith & Smith, 1942), *Cornus* (Wilkinson, 1944) *Lycopersicum* (Cooper, 1927), *Echinocystis* (Miller, 1929), etc. In cases of extreme reduction sepals may not have any vascular supply whatsoever, e. g. in Platanaceae (Boothroyd, 1930), Oleaceae (Joshi & Fotidar, 1941), Fumariaceae (Arber, 1931), etc.

Similar reduction may also be met with in reduced carpels or pistillodes. The sterile carpels of *Grisebachiella,* for instance, do not have any ventral bundles (Woodson, 1935), just as the fertile carpels in certain Thymelaeaceae do not have any dorsals (Heinig, 1951).

2. *Amplification.* In some cases floral organs may receive more than the normal number of vascular traces*. Petals in certain species of the Winteraceae (Nast, 1944), Degeneriaceae (Swamy, 1949), Papaveraceae (Dickson, 1935), Cucurbitaceae (Miller, 1929), etc., receive three bundles each—one dorsal and two marginals. These may be produced by branching of a single petal trace before it enters that organ or they may be derived separately from the stele. In many species of the Compositae the midrib bundles of the petals are replaced by two marginal veins each of which may remain fused with those of the adjacent petals for part or whole of their course (Koch, 1930).

Similarly three-trace stamens occur in the Himantandraceae (Bailey *et al.*, 1943), Degeneriaceae (Bailey & Smith, 1942), Chloranthaceae (Swamy & Bailey, 1950), Winteraceae (Bailey & Nast, 1945), Magnoliaceae (Parkin, 1951), Lauraceae (Reece, 1939), Bamboo (Arber, 1935), etc. In the musaceous *Ravenala* each filament is traversed by from 25 to 28 small vascular bundles, scattered as in the stems of many monocotyledonous plants (Thompson, 1933).

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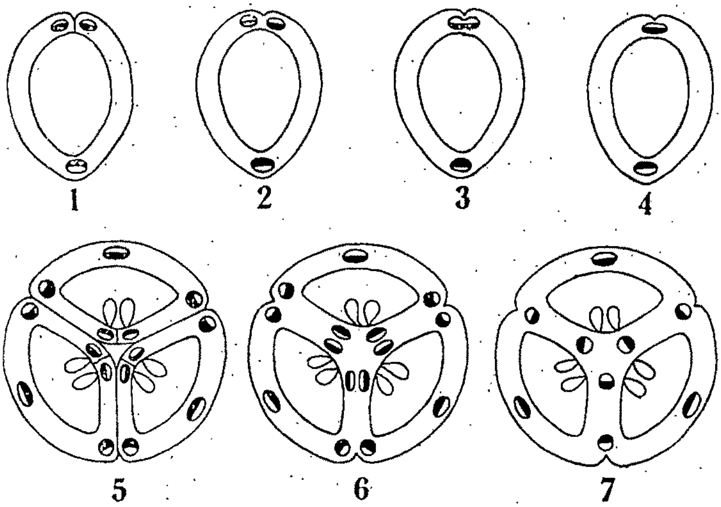
*This does not refer to the several bundles which may be produced by branching of a single bundle in an organ.

Carpels also sometimes may have 5, 7 or more traces per carpel. The larger number of bundles is more often met with in closed carpels with axile placentation. More often the additional bundles branch off from the dorsals but sometimes as in the Caricaceae (Shanti Devi, 1952) they may be derived independently from the parent stele.

The taxonomic significance of this increase in the number of vascular bundles in floral organs is not quite clear. If the ground vascular plan out-lined above also illustrates the most primitive condition, then its modification in either direction should be considered as an advanced feature. And this is believed to be so in the case of carpels (Eames, 1931). But with respect to petals and stamens morphologists in general seem to be in agreement on interpreting the three-trace condition as most primitive and the one-trace condition being derived from it (see Bailey Nast, 1945, Eames and MacDaniels, 1947; Parkin, 1951). This view seems to be based on two factors. In the first place this condition occurs more frequently in some of the most primitive families and secondly this fits in better with the so-called 'old' morphology where stamens and petals are considered as homologues of foliage leaves that are essentially and primitively three trace organs.

These are, however, not quite valid considerations especially from the point of view of those who believe in the telome theory. Even the first point is not of much consequence for all primitive plants may not necessarily be primitive in all respects. Besides, as Wilson (1950) points out these primitive ranalian families themselves exhibit evidence of reduction and specialization in the staminodes of *Degeneria* and *Himantandra,* the single carpel of *Degeneria* and in the number of ovules in *Himantandra* (*see also* Bailey and Nast, 1945). That their stamens have undergone even greater reduction is considered as highly probable by Wilson (1950) who believes that "primitive stamen probably evolved in several directions and underwent numerous modifications. The broad expanded "microsporophyll" of the Degeneriaceae and Himantandraceae is an example of one of these." In these days when there is increasing swing towards polyphylesis it has to be admitted that such a suggestion cannot be ruled out as improbable.

3. *Cohesion.* Cohesion, which refers to fusion between parts of the same whorl, has perhaps been the most active factor in affecting the ground vascular plan. This has affected considerably the number of veins in calyx, corolla and gynaeceum. The number of ribs in the calyx of certain families particularly the Caryophyllaceae and Labiatae is an important taxonomic character. As Eames (1931) has pointed out this is controlled by the intimacy of fusion of marginal veins and the extent of zygomorphy. In *Nipeta veronica*, for instance, all the fifteen veins—three for each sepal—are free throughout their course while in *Ajuga reptans* the marginal veins of adjacent sepals are fused in pairs up to the bases of sinuses.



FIGS. 1–7. Diagrammatic cross sections of typical ovaries, showing structural effects of cohesion, especially upon the vascular bundles. (*After Eames and Mac Daniels, 1947*).

Veronica offers an interesting situation. In a few species the flower is typically pentamerous with five sepals and five petals, while in others the sepals may be 5 or 4, through normal reduction the corolla is only 4-lobed. One of the four lobes is posterior and lies superposed to the sepal which may be in process of vanishing. That this posterior lobe consists of two petals is clearly indicated by its vascular supply in such species as *V. arvensis* where it consists of two mid-rib bundles which give rise to two separate branch systems (Saunders, 1939). Similar instances of "loss" of parts through fusion have also been reported in some species of *Cistus* and *Hypericum* (Saunders, 1939).

In the case of ovary, cohesion has resulted in much greater complexities in the vascular ground plan. Not only the carpellary ventrals and median laterals show various stages of fusion in pairs (*see* figs. 1-7), but sometimes the fusion is so 'deep-seated' that two bundles arise as one from the parent stele. In the achenial carpels which are believed to be derived from follicle types, the vascular supply has been reduced to the simplest. In *Ranunculus aquatilis,* for instance, the single sub-basal ovule *appears* to be borne on 'dorsal' bundle, this situation having been brought about by complete fusion of ventrals to the dorsal and being clear through a study of the less reduced species (Chute, 1930).

4. *Adnation.* Vascular bundles of organs of different whorls may fuse together if they happen to lie close to one another radially or tangentially. The commonest example is perhaps that of the sepal marginals which in many cases arise conjoint with petal dorsals. Sometimes two sepal marginals, one petal midrib and one staminal bundle may all arise as a single bundle which in the receptacular cortex separates into its component parts (*see* Eggers. 1935 ; Norris, 1941 ; etc.). In cases of extreme compression even the carpellary dorsals also arise as fused with the sepal-petal-stamen strand. Such cases are often seen in the 'wall' of the inferior ovary.

In *Polanisia trachysperma* (Capparidaceae), which is apparently zygomorphic and tetramerous with four sepals and four petals, the abaxial sepal is larger than the others and its vascular supply is equivalent to that of two normal sepals and one petal (Stoudt, 1941). This is interpreted as a fusion of two sepals and one petal into a single large "sepal" and strongly suggests a pentamerous ancestral form with radial symmetry.

A highly complex situation is reported in *Saraca indica,* where the four perianth segments have between them three, three, two, two, or ten vascular bundles which in a typical leguminous flower supply two perianth whorls of five each (Saunders, 1939). This is interpreted to mean that all the ten perianth leaves are present and that they are fused into four groups. Following the same analogy further, Saunders has interpreted the corolla in Primulaceae and certain Sapotaceae and Theophrastaceae as a "compound" structure in which, in addition to the petals, are incorporated more or less completely five antesepalous staminodes. As has already been pointed out (Puri, 1951), there is, however, little justification for such an interpretation.

A unique case of cohesion and adnation is reported in *Villarsia ovata* (Menyanthaceae), where all bundles for different floral whorls fuse completely into a vascular cylinder in the ovary wall (Lindley, 1938).

When stamens are epipetalous and antepetalous, their bundles many arise conjoint with petal midrib bundles and may not separate from them for varying distance. An interesting example of adnation is offered by *Aristolochia*, where the staminal bundles are adnate to the carpellary bundles right up to the top of the ovary. This was once made the basis for the erroneous suggestion that the stigma here is of staminal origin (*see* Puri, 1951).

Adnation is frequently referred to in connection with the formation of "floral cups" and "wall" of the inferior ovary. The present author is inclined to believe that freedom or gradual adnation of vascular bundles of different floral whorls is of little consequence in determining the morphological nature of the "floral cups" or the "wall" of the inferior ovary. Those who have depended upon vascular bundles for the solution of such problems have apparently failed to appreciate the dual character of a bundle—the "trace" part that traverses the receptacular cortex and the "bundle" part that enters the organ (*see* Puri, 1952 b).

CONTRIBUTION OF FLORAL ANATOMY TO TAXONOMY

Let us now refer briefly to some works on floral anatomy which throw some light on the systematics of certain groups, a complete citation of such works being obviously outside the scope of this paper.

Bailey and his associates have recently made a detailed morphological study of the so-called Magnoliaceae and they have now rearranged this vast assemblage under several distinct families. Regarding the position of *Drimys* in the Magnoliaceae special tribe (Illicieae DC., Wintereae R. Br.) or subfamily (Drimyoideae Harms, Drimydoideae Skottsb.), Bailey and Nast (1945) have reached the conclusion that it is wholly untenable. They have justifiably raised the genus to the status of a full family, Winteraceae, comprising six genera—*Drimys, Bubbia, Belliolum Pseudowintera, Exospermum* and *Zygogynum*. They have been found to differ from the Magnoliaceae (*sensu restrictus*) in important morphological characters of the pollen, stamens, carpels, perianth, secondary phloem, nodal anatomy, vascularization of leaf, floral organs and receptacle. Whatever similarity is there between the carpels of *Degeneria* and of Sec. *Tasmannia* of *Drimys* is believed to be "apparently due to the retention of primitive ranalian megasporophylls by plants which exhibit fundamentally different trends of morphological specialization in their flowers, stems and leaves." They finally conclude that "although the Winteraceae obviously are of general ranalian affinities, ..., they do not appear to be closely related to any specific surviving family of the ranalian complex."

On more or less similar summation of evidence from both vegetative and reproductive organs Bailey and his associates have distinguished Himantandraceae (*Himantandra*), Degeneriaceae (*Degeneria*), Magnoliaceae (*sensu restrictus*) (*Magnolia, Liriodendron*, etc.) Trochodendraceae (*Trochodendron* and *Tetracentron*), and Schizandraceae (*Schizandra* and *Illicium*).

Among the other ranalian families Ranunculaceae perhaps has been most thoroughly investigated especially from the point of view of floral anatomy. The exhaustive works of Broulan (1935), Smith (1926, 1928) and Chute (1930) have provided much useful information about this interesting group. Brouland (1935) on the basis of his very extensive anatomical investigation divides the family into four tribes: 1. Helleboreae, 2. Anemoneae, 3. Ranunculeae and 4. Paeonieae. His separation of *Ranunculus* (included in Anemoneae by Prantl) into a separate tribe is significant and perhaps welcome. For this he has depended primarily upon the vascular supply of the carpel and that of the ovule. Earlier, Smith (1926) also had drawn attention to the unique features of reduction and specialization of the carpel in this genus.

Chute (1930) has drawn attention to a very significant point of distinction between the achenial carpels of the Rosaceae and those of the Ranunculaceae. In the former she has observed a greater reduction in the dorsal bundle than in the ventral bundles while in the latter it is the ventrals which have suffered the greatest reduction. Besides, she has brought forward interesting evidence for interpretting the achnes as reduced follicles, all stages of reduction from the typical three-trace follicle to the most simple one-trace achene having been observed.

In addition to the Ranunculaceae, Smith (1928) has also studied some members of the Menispermaceae, Calycanthaceae and Anonaceae and he concludes that the vascular skeleton of the flower of each gives undoubted evidence of their having originated from the Ranunculaceae.

Chapman (1936) having studied the carpel anatomy and the placental peculiarities of the Berberidaceae has attempted to rearrange the genera with a view to show their relationships. She distinguishes two lines of evolution in this family which she believes to have evolved side by side with the Ranunculaceae from a pro-ranalian complex.

The so-called Amentiferae and other allied families have attracted much attention from floral anatomists. Many papers have appeared on these families from time to time (for literature see Puri, 1951). Without going into details of these it may be pointed out that two points become abundantly clear from these studies. In the first place the group is a very heterogenous one and secondly the various families are more are less specialized and that their simple appearance is due to reduction. These points obviously are of great significance to the systematist.

The rhoeadalean flower in general and that of the Cruciferae in particular has long engaged the attention of floralan atomists. Like students of comparative morphology, they have often tried to slove the so-called problem of the number in the rhoeadalean gynaecea. The present author also has been actively interested in this group for more than fifteen years (*see* Puri, 1941, 1942, 1945, 1950, 1951, 1952 a, 1952 c) and he was now reached the conclusion that the gynaeceum make-up in the Papaveraceae, Capparidaceae, Cruciferae and Moringaceae is essentially on the same plan. In all of them, although the placentation is parietal, the placental bundles are inverted with reference to the mother axis and occur on the inner side of the secondary marginal strands with normal orientation. This has been made the basis for the suggestion that parietal placentation in these families has been derived from axile placentation and that during the change the original inverse orientation of the placental strands has somehow been retained. This is perhaps the simplest explanation of the peculiarity of the placental region in these families and if this is accepted we need not postulate the participation of a larger number of carpels than is usually believed to be the case. Further this structural feature strengthens considerably the inclusion of the doubtful Moringaceae in the Rhoeadales. And as this peculiarity has also been reported in the Passifloraceae and Cucurbitaceae (Puri, 1947, 1952 a) it supports the suggested affinities between the Moringaceae and the Passifloraceae.

Dickson (1935), who has studied *Glaucium* and other genera of the Papaveraceae in great detail, has drawn some tentative conclusions regarding the general position of the family and the arrangement of various genera within it. As most of these conclusions are based on the conception of "expanded" and "contracted" carpels—which conception is no more valid as far as the present author is concerned (*see* Puri, 1950, 1952 c)—they need further verification. However, her remarks that the family shows great plasticity and that it is directly connected with the Ranunculaceae through *Platystemon* are worthy of note.

Norris (1941) has made a detailed study of torus anatomy and nectary characteristics in Resedaceae, Papaveraceae, Fumariaceae and Cruciferae. On account of total absence of nectaries in the tori of the Papaveraceae he thinks that this family cannot be regarded as having given rise to any other family of the Rhoeadales as is generally believed to be the case. Resedaceae and Capparidaceae are considered to be the most primitive families of the existing Rhoeadales and derived probably by more or less parallel evolution "from a common ancestral group without connections between sepal bundles and petal bundles in the tori and with annular nectaries." He further assumes that the Papaveraceae, Fumariaceae and Cruciferae were derived by subsequent parallel evolution from a common ancestral group somewhat resembling the existing Resedaceae and Capparidaceae.

Stoudt (1941), on the basis of the structure of perianth, nectary and pistil*, suggests a close relationship between the Capparidaceae and Cruciferae. This is against Hutchinson's separation of the Capparidaceae into a separate order.

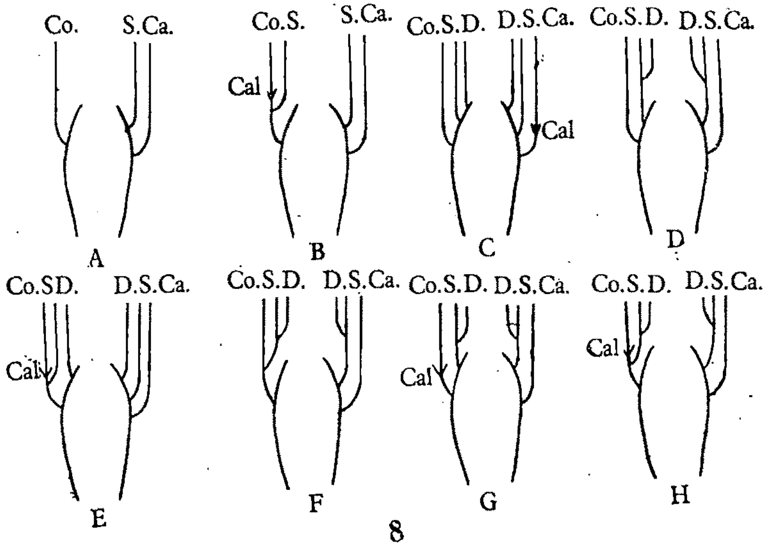


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FIG. 8. Diagrams showing the vertical relationships of the vascular traces in the flowers of various Rutaceae. A. *Diosma succulents* Berg., *Xanthoxylum fraxineum* Willd., *Toddalia aculeata* Pers. *Feronia elephantum* Correa, *Aegle sepiaris* DC. B. *Cneoridium dumosum* Hook. C. *Ruta bracteosus* DC., R. *graveolens* L., *Citrus aurantium* L. E. *Adenandra uniflora* Willd., *Berosma crenulata* Hook.. *Calodendrum capensis* Thunb. F. *Agathosma imbricata* Willd G. *Boronia fastigiata* Bartl.. *B. heterophylla* F. Muell., *B. megastigma* Nees. H. In *Dictamnus fraxinella* Pers. (Ca=Calyx trace. Cal=Calyx lateral trace ; Co=petal trace ; D=disc trace ; S=stamen trace). (*After Moare, 1936*).

Moore (1936), who has analysed Saunders' data on Rutaceae has found eight different sorts of anatomical conditions which differ in (1) adnation of traces (2) cohesion of traces (3) inserion of stamen traces and (4) insertion of disc traces (fig. 8,

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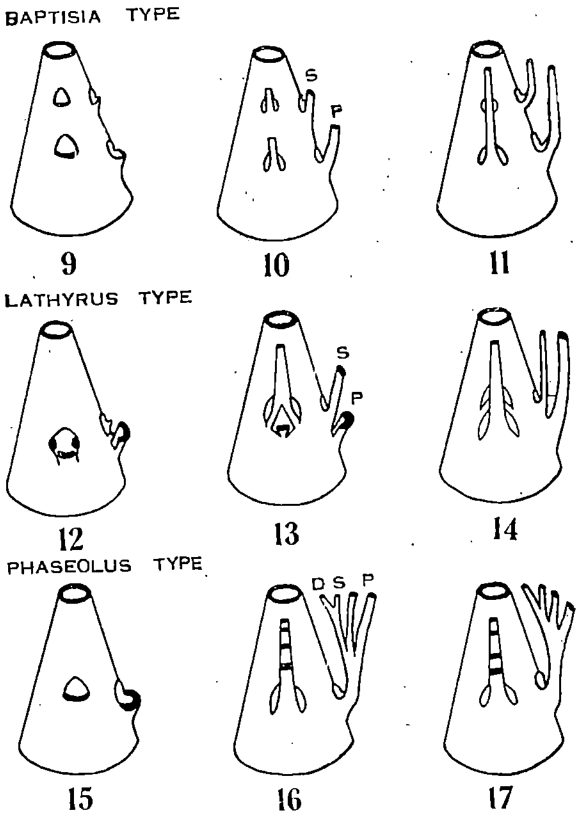
*Stoudt believes in the 2n-carpellary view for capparid gynaeceum, which is shown to be incorrect (Puri, 1950).

A.H). Type A is believed to be the simplest and Type H the most complex. The sub-family Rutoideae exhibit seven of the eight types and is, therefore, considered to be most heterogenous phylogenetically. *Ruta* and *Citrus*, which are generally placed rather far apart by systematists, show rather similar floral anatomy (Type C & D). It is significant to note that Barner's (1927) observations of serodiagnosis agree remarkably well with those of floral anatomy. On the other hand *Ruta* and *Dictamnus*, which are placed very near to one another by taxonomists, show different floral anatomy (types C & H). Further information, alone can decide which is the correct treatment.

Heinig (1951) has worked out the vascular anatomy of the flower of the Thymelaeaceae and she concludes that the family as a rule is apetalous (petals occurring in some primitive members), the petaloid scales being just stipular outgrowths from sepals as they get this vascular supply branched off from sepal traces. Regarding the systematic position of the family Heinig writes: "The polypetalous and polystamenous condition of the primitive members of the group and the modified parietal placentation suggest a derivation of the family from polymerae parietalean family such, perhaps, as the Flacourtiaceous, which in syngamy and the possession of supernumerary scales further resembles the Thymelaeaceae."

Moore's (1936 b) study of vascular anatomy of the flower of certain species of the Papilionatae not only sustains the well-established classification of the family but also brings to notice new characters of great taxonomic value. Notwithstanding the apparent uniformity in the organization of the papilionaceous flower, the vascular ground plan shows definite and significant trends. Moore distinguishes two anatomical series according to the nodal relationships of the traces to the sepals and petals and to the stamens. These are designated as (1) Dihiate (Hiatus, meaning gap or cleft) series and (2) Unihiate series. In the Dihiate series there are two sets of traces (and gaps) which supply calyx, corolla and stamens. This is further distinguished into (a) *Baptisia* type and (b) *Lathyrus* type. All genera studied by Moore from Sophoreae, Podalyrieae, Genisteae fall under *Baptisia* type (figs. 9-11) and those from Trifolieae, Loteae, Galegeae, Hedysareae, Dalbergieae and Vicieae under *Lathyrus* type (figs. 12-14). In the Unihiate series, on the other hand, there is one set of gaps from which one set of traces depart which later on split into calyx, corolla and stamen traces (figs. 15-17). Under this there is only the *Phaseolus* type that includes all genera studied in Phaseoleae. This is further characterized by the possession of a staminodal disc—a character which has not been taken advantage of by taxonomists—and climbing habit.



FIGS. 9–17. Diagrams illustrating the salient features of the floral anatomy of the Papilionatae. Carpellary traces not shown. *Baptisia* type.—Two nodes, perianth (P), and staman (S), separated by a relatively long internode. *Lathyrus* type.—The internode separating perianth and androecium is greatly shortened. *Phaseolus* type.—The trace to the floral appendages on the same radius are completely fused and depart from the stele as one large strand. D=disc trace. (*After More, 1936*).

Smith and Smith (1942) have described in detail the vascular anatomy of the flower in a number of Santalaceae, Olacaceae and other related families. They have observed a "downturning" in the course of vascular bundles in the "wall" of the inferior ovary both in the Santalaceae and Olacaceae. On the basis of this important anatomical feature they have concluded, perhaps rightly, that the inferior ovary here is of "axial" nature and that the two families are most closely related together. Their inferences regarding the other families, where they interpret the inferior ovary as "appendicular" need varification (*see* Puri, 1952 b).

In 1936 three talented ladies, whose names begin with D, dealt with the affinities of pinks and primroses in the American Journal of Botany (a great coincidence !). They all agreed on this point that the free central placentation in pinks and primroses has been derived in the same manner, the Primulaceae being just one evolutionary stage in advance of the Caryophyllaceae. Their conclusions are graphically shown in fig. 18. Dawson (1936) whose work appeared the last made a comparative anatomical study of the flower of Polemoniaceae and Caryophyllaceae with a view to determine their relationships. She arrived at the conclusion that the Polemoniaceae have been derived from a caryophyllaceous stock having three carpels, before free central placentation was established as a family character. Evidence for such an inference is found in the general vascular scheme of the flower and more particularly in the behaviour of the carpellary bundles in the two families. Her criticism that Geraniaceae should not be treated as an intermediate family between the Primulaceae and the Caryophyllaceae, as Dickson has done, appears to be justified for they never had free central placentation.

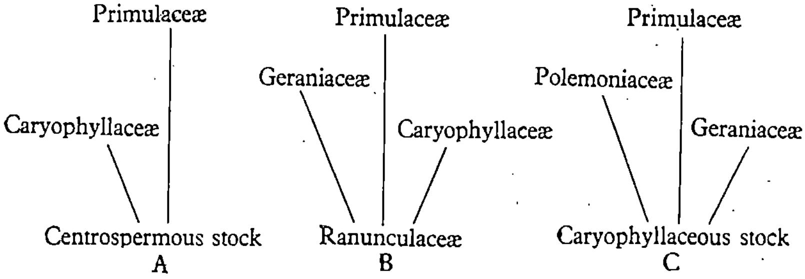


FIG. 18. Graphical representation of the relationships of the Primulaceae with other families. (A. B. C. *based respectively on the inference of Douglas 1936, Dickson 1936 and Dawson 1936*).

Lindsey (1938) has brought to light important anatomical features in floral morphology which more than validate the separation of Menyanthaceae from the Gentianaceae. The most important distinction that he discovered is with respect to the fusion of vascular bundles. While in the advanced genera of the Gentianaceae vascular traces in the receptacle fuse to form distinctive concentric bundles, in the Menyanthaceae this fusion is more general and forms "a definite horizontal ring by cohesion and adnation." Besides this other floral features which distinguished this group from the Gentianaceae are : (1) presence of ovule traces, (2) fusion of adjacent corolla laterals, (3) bilaterally symmetrical vascular pattern, and (4) epigynous insertion of floral parts in certain genera, accompanied by extreme vascular fusion.

The thus circumscribed Gentianaceae has been subjected to a further detailed study by Lindsey (1940). Here he justifies the division of the genus *Gentiana* into two subgenera, *Eugentiana* with separate carpellary ventrals and *Gentianella* with strong original fusion of carpellary ventrals. Further, he has found that the simplest anatomy with a minimum of vascular fusion is met with in the simplest subtribe Erythraeinae.

Palser (1951) has offered a tentative grouping of the species of Andromedeae (Ericaceae) studied by her. The first separation is based on the extent of fusion of sepal bundles. For further subdivisions, however, she has depended upon other features such as presence or absence of fibres with sepal traces, position of ventral carpel bundles, adnation and cohesion of traces of different organs, etc.

Copeland (1943), working on Rhododendroideae, experienced that various characters of the vascular anatomy of the receptacle tend to mark tribes but do not serve for precise definitions.

In the Apocynaceae the vascular systems of flower, particularly those of the calyx do not, in general, support the existing classification nor do they help in the formulation of a new system. Woodson and Moore (1938) therefore have reached the conclusion that in Apocynaceae the results of a study of vascular anatomy of the flower "appear to be of little value to pure taxonomy."

Lawrence's (1937) study of the vascular anatomy of the Boraginaceae, on the whole, confirms the taxonomic treatments of the subfamilies and tribes. The Cordioideae and certain species of the Ehretioideae, however, appear to "represent a branch of development distinct from the rest of the family in the possession of fused ventral traces."

Having studied the carpellary and placental structure in the Solanaceae, Murray (1945) has reached the conclusion that in general, the floral anatomy furnishes additional evidence of the validity of Wettstein's arrangement of the genera. She thinks that it would be better to place the Hyocyamenae at the end of the Solanaceae because the flowers of *Hyocyamus* are slightly irregular.

On the basis of his extensive anatomical investigations on the Cornaceae and other related families, Horne (1914) concludes : "The floral vascular systems of the Cornaceae do not conform to one type as in the Caprifoliaceae. It is in *Cornus* of the Caprifoliacean type. The systems of *Davidia* and *Marlea* are transitional in character between that found in *Aralia* and the synthetic systems of *Cornus* and the Caprifoliaceae." He, therefore, asserts that the Cornaceae are polyphyletic, certain genera having pursued independent but similar lines of development. Wilkinson (1944, 1949) also appears to have arrived at a more or less similar inference with regard to the affinities of the Cornaceae with the Caprifoliaceae. On the consideration of vascular supply of the ovule, she finds greatest resemblance between the subfamily Cornoideae and *Viburnum* and *Sambucus* (she justifies the retention of *Sambucus* in the Caprifoliaceae) of the Caprifoliaceae.

A study of the vascular anatomy of the flower of Compositae, though not of much consequence in determining relationships of the family, has helped much in elucidating floral morphology. Koch (1930), for instance, has shown that the ligulate and bilabiate corollas have been derived quite independently from tubular corolla and that the bilabiate corolla is not an intermediate stage as has sometimes been suggested. Further, the calyx lobes with their median bundles are believed to have become fused completely with the corolla of ray florets in Heliantheae. Here, therefore, the so-called pappus does not represent calyx. In some respects these anatomical observations appear to go counter to the findings of taxonomists. Heliantheae, for instance, which is usually regarded as fairly advanced, is primitive in retaining the median bundles of the petals which are lost in all other tribes. Again, *Aster* and *Senecia* are far separated taxonomically but from anatomical point of view they are closely related in having reduced vascular supply (Koch, 1930). However, in determining relationships much reliance cannot be placed on single characters.

Comparatively few monocotyledonous families have so far attracted the attention of floral anatomists. Blaser (1941) has studied a number of the Cyperaceae and as a result of his finding a more or less uniform vascular plan in all the species studied, he has reached the conclusion that the family is a unified group. He also finds anatomical evidence for interpreting the single basal ovule as having been derived from axile or free central ancestry. This is considered to be an important distinction from the Gramineae where the ovule is excentric and derived from parietal ancestry.

Among the monocotyledons, the Gramineae, and among the dicotyledons, the Parietales and the Centrospermae, are some of the orders which can well bear detailed anatomical investigations. Work is in progress on some of these in this laboratory, but it requires the efforts of many workers in tropical countries before we see clearly through these rather confused assemblages.

CONCLUSION

From this brief review it is apparent that a comparative study of the floral anatomy of a group does help the systematist by revealing the extent of specialization of the various members of their flower. But it must be borne in mind that all characters of a species do not evolve at the same rate. Some may not change at all for some time or may change in opposite direction. Moreover, changes vary in their significance both in themselves and in comparison with different species. In assessing the relative progress of the various species, therefore, we have to take into account the sum total of various advanced traits together with their significance (C. F. Wilkinson 1944). While doing so we cannot possibly ignore the probable role played by parallel evolution. Thus the whole thing becomes very complicated and we have got to exercise utmost care in analysing our data for the benefit of the systematist.

The author is thankful to Smt. Shanti Devi, M.Sc., for drawing the illustrations for this paper.

LITERATURE CITED

Abbe, E.C. and T. T. Earle, 1940. Inflorescence, floral anatomy and morphology of *Leitneria floridana. Bull. Torrey Bot. Club*, **67** : 173-193.

Arber, A. 1950. The natural philosophy of plant form. Cambridge.

Bailey, I.W. and C.G. Nast, 1945. The comparative morphology of the Winteraceae. VII. Summary and conclusion. *J. Arnold Arb.*, **26** : 37-47.

Bailey, I. W., C. G. Nast and A. C. Smith. 1943. The family Himantandraceae. *J. Arnold Arb.*, **24** : 190-206.

Bailey, I.W. and A.C. Smith. 1942. Degeneriaceae, a new family of the flowering plants from Fiji. *J. Arnold Arb.*, **23** : 356-366.

Bailey, I.W. and B.G. L. Swamy 1949. The morphology and relationships of *Austrobaileya. J. Arnold Arb.*, **30** : 211-226.

Blaser, H.W. 1941. Studies in the morphology of the Cyperaceae. I. A & B. *Amer. J. Bot.*, **28** : 542-551 and 832-838.

Boothroyd, L.E. 1930. The morphology and anatomy of the inflorescence and flower of the Platanaceae. *Amer. J. Bot.*, **17** : 678-693.

Broulecd, M. 1935. Recherches sur l' anatomie florale des Ranunculacees. Thesis. Univ. Paris.

Camp, W.H. 1940. The concept of the genus. V. Our changing generic concepts. *Bull. Torrey Bot. Club*, **67** : 381-389.

Chaqman, M. 1936. Carpel anatomy of the Berberidaceae, *Amer. J. Bot.*, **23** : 340-348.

Chute, H. M. 1930. The morphology and anatomy of the achene. *Amer. J. Bot.*, **17** : 703-722.

Copeland, H.F. 1943. A study, anatomical and taxonomic, of the genera of Rhododendroideae. *Amer. Mid. Nat.*, **30** : 533-625.

Cooper, D.C. 1927. Anatomy and development of tomato flower. *Bot. Gaz.*, **83** : 399-411.

Dawson, M.L. 1936. The floral morphology of the Polemoniaceae. *Amer. J. Bot.*, **23** : 501-511.

Devi, Shanti 1952. Studies in the order Parietales. III. Vascular anatomy of the flower of *Carica papaya* L. With special reference to the structure of the gynaeceum. *Proc. Indian. Acad. Sci.*, (In Press).

Dickson, Jean. 1935. Studies in floral anatomy. II. The floral anatomy of *Glaucium flavum* with reference to other members of the Papaveraceae. *J. Linn. Soc. (Bot.)*, **50** : 175-224.

Dickson, J. 1936. Studies in floral anatomy. III. An interpretation of the gynaeceum in the Primulaceae. *Amer. J. Bot.*, **23** : 385-393.

Douglas, G. 1936. Studies in the vascular anatomy of the Primulaceae. *Amer. J. Bot.*, **23** : 199-212.

Eames, A.J. 1931. The vascular anatomy of the flower with refutation of the theory of carpel polymorphism. *Amer. J. Bot.*, **18** : 147-188.

Eames, A. J. and L. H. Mac Daniels. 1947. An introduction to plant anatomy. Sec. Edi. Mc Graw-Hill Book Co., New York.

Eggers, Otto 1935. Ueber die morphologische Bedeutung des Leitbundelverlaufes in der Bluten de Rhoeadalen und ueber des Diagram der Cruciferen und Capparidaceen. *Archiv-Wissensch.*, **24** : 14-58.

Heinig, Katherine H. 1951. Studies in the floral morphology of the Thymelaeaceae. *Amer. J. Bot.*, **38** : 113-132.

Horne, A.S. 1914. A contribution to the study of the evolution of the flower, with special reference to the Hamamelidaceae, Caprifoliaceae, and Cornaceae. *Trans. Linn. Soc. Lond.* (2) **8** : 239-309.

Joshi, A.C. and A.N. Fotidar. 1941. Floral anatomy of *Olea fragrans* Thumb. *Proc. Nat. Inst. Sci.*, **7** : 369-375.

Kasopligil, Baki. 1951. Morphological and ontogenetic studies of *Umbellularia californica* Nutt. and *Laurus nobilis* L. *Univ. Calif. Pub. Bot.*, **25** : 115-240.

Koch, Minna F. 1930, Studies in the anatomy and morphology of the composite flower. I & II. *Amer. J. Bot.*, **17** : 938-952 & 995-1010,

Lawrence, Julia Ruth. 1937. A correlation of the taxonomy and the floral anatomy of certain of the Boraginaceae. *Amer. J. Bot.*, **24** : 433-444.

Lindsey, Alton A. 1938. Anatomical evidence for the Menyanthaceae. *Amer. J. Bot.*, **25** : 480-485.

Lindsey, Alton A. 1940. Floral anatomy in the Gentianaceae. *Amer. J. Bot.*, **27** : 640-652.

Miller, W.L. 1929. Staminate flower of *Echinocystis lobata*. *Bot. Gaz.*, **88** : 262-284.

Moore, J. A. 1936. Floral anatomy and phylogeny in the Rutaceae. *New Phyt.*, **35** : 318-322.

Moore, J.A. 1936b. The vascular anatomy of the flower in the papilionaceous Leguminosae. 1 & 2. *Amer. J. Bot.*, **23** : 279-290 & 349-355.

Murray, Mary Aileen. 1945. Carpellary and placental structure in the Solanaceae. *Bot. Gaz.*, **107** : 243-260.

Nast, C.G. and I.W. Bailey. 1945. Morphology and relationship of *Trochodendron* and *Tetracentron*. II. Inflorescence, flower and fruit. *J. Arnold Arb.*, **26** : 265-276.

Nast, C.G. and I.W. Bailey. 1946. Morphology of *Euptelea* and comparison with *Trochodendron*. *J. Arnold Arb.*, **27** : 186-192.

Norris, Theodore. 1941. Torus anatomy and nectary characteristics as phylogenetic criteria in the Rhoeadales. *Amer. J. Bot.*, **28** : 101-113.

Palser, Barbara F. 1951. Studies of floral morphology in the Ericales. I. Organography and vascular anatomy in the Andromedeae. *Bot. Gaz.*, **112** : 447-485.

Parkin, John. 1951. The protrusion of the connective beyond the anther and its bearing on the evolution of the stamen. *Phytomorphology*, **1** : 1-8.

Puri, V. 1951. Role of floral anatomy in the solution of morphological problems. *Bot. Rev.*, **17** : 271-353.

Puri, V. 1952a. The Flower : 3. Placentation in the rhoeadalean flower. Agra Univ. Exten. Lecture, Agra.

Puri, V. 1952b. Floral anatomy and inferior ovary. *Phytomorphology*, **2** : (In Press).

Puri, V. 1952c. Placentation in Angiosperms. *Bot. Rev.* **18** : (In Press).

Saunders, E.R. 1939. The neglect of anatomical evidence in the current solution of problems in systematic botany. *New Phyt.*, **38** : 203-209.

Smith, F.H. and F.C. Smith. 1942. Floral anatomy of the Santalaceae and related forms. *Oregan State Monographs, Studies in Botany*, 5.

Smith, Geo. Hume. 1926. Vascular anatomy of Ranalian flowers. I. Ranunculaceae. *Bot. Gaz.*, **82** : 1-29.

Smrte, Geo. Hume. 1928. Vascular anatomy of Ranalian flowers. II. Ranunculaceae (contd.) Menispermaceae, Calycanthaceae, Anonaceae. *Bot. Gaz.*, **85** : 152-177.

Stoudt, Harry N. 1941. The floral morphology of some of the Capparidaceae. *Amer. J. Bot.*. **28** : 664-675.

Swymy, B.G.L. 1949. Further contributions to the morphology of the Degeneriaceae. *J. Arnold Arb.*, **30** : 10-38.

Thompson, J. Mcoeln. 1933. Studies in advancing sterility. VI. The theory of scitaminean flowering. *Univ. Liverpool, Hartley Bot. Lab. Pub.* **11**, : 1-114.

Wilkinson, A.M. 1944. Floral anatomy of some species of *Cornus. Bull. Torrey Bot. Club.*, **71** : 276-301.

Wilkinson, A. M. 1949. Floral anatomy and morphology of *Triosteum* and of the Caprifoliaceae in general. *Amer. J. Bot.*, **36** : 481-489.

Wison, C.L. 1950. Vasculation of the stamen in the Melastomaceae with some phylogenetic implications. *Amer. J. Bot.*, **37** : 431-444.

Woodson, R.E., Jr. 1935. The floral anatomy and probable affinities of the genus *Grisebachiella* Lorentz. *Bull. Torrey Bot. Club*, **62** : 471-478.

Woodson, Jr. R.E. and J. A. Moore. 1938. The vascular anatomy and comparative morphology of apocynaceous flower. *Bull. Torrey Bot. Club.*, **65** : 135-166.

Note : Where several papers by the same author are referred to, full citation of the earlier ones will be found in the titles cited here.

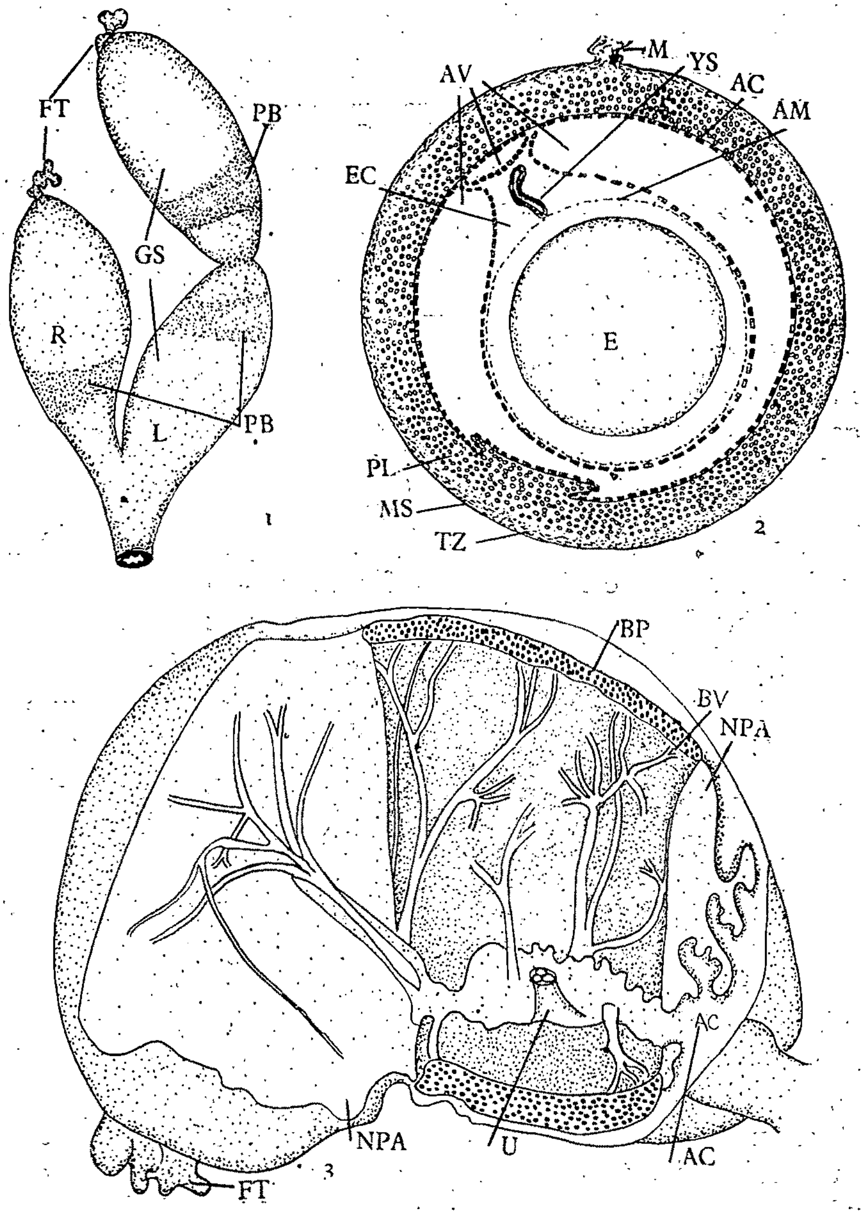
CONTRIBUTIONS TO OUR KNOWLEDGE OF THE STRUCTURE OF THE PLACENTA OF *PANTHERA TIGRIS TIGRIS* (LINN.)

By Shyama Charan Srivastava, M. Sc., Ph. D., *Reader in Zoology, Victoria College, Gwalior.*

The placental structure of *Panthera tigris tigris* (Linn.) has not yet been studied. The only work on an allied species of the family is the rather sketchy account by Robinson (1903) on *Panthera pardus* (Linn.). The present study is based on material placed at my disposal by the courtesy of Raja Saheb Panchum Singh of Pahargarh, Gwalior. A tigress shot at 10 in the previous night was skinned in the usual course and the three gestation sacs that were found in it were injected with 20% formalin at 8 next morning and were left in the same fluid for 24 hours. Part of the placenta and foetal membrances were then transferred to bouin's fluid for 96 hours, with a change of the fluid every 24 hours and the remaining material was stored in 10% formalin. The material fixed in bouin's fluid was washed in 70% alcohol and preserved in 90% alcohol. I take this opportunity of expressing my grateful thanks to the Raja Saheb for his kindness in placing the specimens at my disposal.

The Gestation Sacs.—The bicornuate uterus contained a single gestation sac in the right horn and two gestation sacs in the left horn. The 3 gestation sacs together weighed 9 lb. and measured about 10·5 × 5 × 4 inches. The cranio-caudal direction of the foetuses and the position of the placental ring differ. On the right side, the cranial end of the foetus is on the cranial side of the mother and the placental ring close to the posterior pole of the sac. On the left side, the cranial end of the anterior foetus faces the caudal side of the mother and possesses the placental ring on the same side, while the cranial end of the posterior foetus faces the cranial end of the mother and bears the placental ring close to the anterior pole of the sac. Thus the orientation of the placenta in

Panthera tigris shows an important departure from the normal equatorial position in *Felis, Canis, Herpestes,* etc.



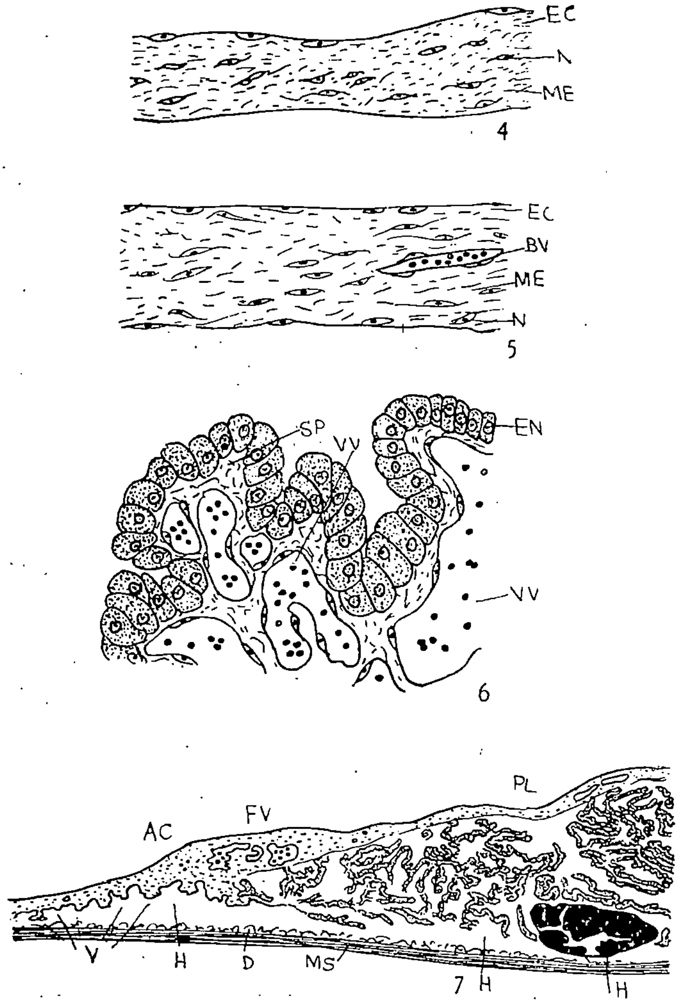
FIGS. 1—3. Placentation in *Panthera tigris tigris*. 1. Three gestation sacs ; 2. Foetal membranes : 3. Gestation sac dissected to show the placental belt.

VASCULARISATION OF THE SACS.—The gestation sacs are vascularised by the genital vessels, which spread over the sacs from the side of the mesometrium. The blood vessels are more distinct in the portions having placenta internally and (b) the blood vessels are more notable on the outer side than on the inner side of the sacs. This is possibly due to the pressure on the blood vessels caused by the contact of the sacs towards their inner sides.

FOETUS.—The foetus for the purpose of the following description, is taken out of the right gestation sac. This being near term is fully formed. The body is brownish-yellow and is deeper dorsally than ventrally. The black stripes are distinct. The whole body is clothed by a soft fur, except on the palm and pes. The cranio-caudal length is 18.5 inches and the head hangs vertically from the spine. The back of the head, neck and the trunk together form a convex outline and the tail is bent twice on itself. The foetus in the left anterior gestation sac is male and that in the posterior female.

The umbilical cord is oval in transverse section and measures 10 × 8 mm. just at the base. It possesses a large central artery, two peripheral veins and a yolk-sac stalk. These are enclosed in the amnion. The umbilical artery, at a distance of 12 mm. approximately from the base divides into two parts and thus the allantoic vesicle on each side of the foetus is supplied with an artery and a vein.

FOETAL MEMBRANES.—The amnion loosely encloses the foetus. It is adherent to the umbilical cord, is free from the neighbouring membranes and underlines the inner wall of the allantoic vesicle in the mesometrial third of the sac and is adherent to the inner wall of the allantoic vesicle in the remaining two-thirds of the sac. This coherence of amnion with the inner wall of the allantois has also been noted in dog by Bischoff (1845) and in cat by Otto Grosser (1910). The chorion, as in all carnivores, joins the outer wall of the allantoic vesicle through mesoderm and forms the allantochorion. The yolk-sac is greatly reduced and laterally compressed. This sac extends cranio-caudally in the exocoelome on the mesometrial side and inhibits the union of the allantoic vesicles from the left and right sides of the gestation sac. The cavity of the allantoic vesicle is wide and its union with other membranes has already been stated above.



FIGS. 4—7. Plancentation in *Panthera tigris tigris*. 4. Section of amnion ; 5. Section of allanto-amnion ; 6. Section of yolk-sac ; 7. Section showing the dried and greatly reduced haematomes.

The layer of amnion is 0·03 mm. thick and is composed of inner ectoderm and outer mesoderm but does not show any vascularisation. The cells in the ectoderm (Fig. 4) are highly flattened, with elongate nuclei and thickly granular cytoplasm, the cells in the mesenchyme are like those in the ectoderm but with numerous intercellular spaces. In the region where amnion has united with the inner layer of the allantoic vesicle, the total thickness of the two membranes is 0·04 mm. and fine blood capillaries are noticeable.

The inner wall of the yolk-sac is greatly folded on itself and consists of small cuboidal cells with centrally situated, basiphilic and spherical nuclei and lightly eosinophilic and granular cytoplasm. The outer wall of the sac is mesenchymatous and consists of scattered stellate cells and vitelline vessels. These contain enucleated red blood corpuscles.

The innermost layer (endoderm) of the outer wall of the allantoic vesicle is of flattened cells with elongate nuclei and finely granular cytoplasm. The mesenchyme shows loose masses of stellate cells and a number of foetal vessels. This mesenchyme forms the core of the villi and is distinct on account of its typical structure and its separation from the syncytiotrophoblast. The outermost layer of the allantochorion is syncytial and trophoblastic. It invests the villi and encloses the maternal vessels in the placental labyrinth and underlies the detritus on the tops of villi. The nuclei in this syncytium are relatively small and the cytoplasm present between these and the maternal endothelia is thickly granular. The thickness of this cytoplasm is moderate, being less than what it is in dog.

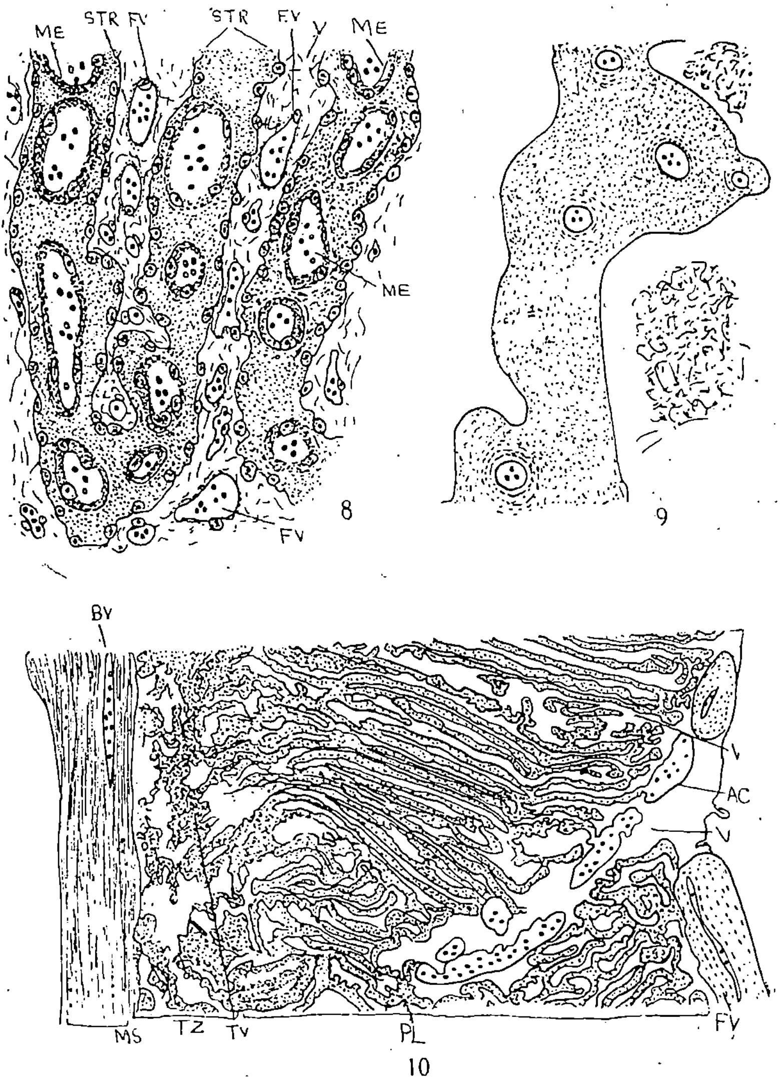
PLACENTA.—The placenta (Fig. 3) is zonary in type as in cat, dog and mongoose, but as already stated, either near the cranial or the caudal end of the gestation sac (Fig. 7). The width of the placental belt varies from 7 to 12 cm. and is thus unequal. *Panthera tigris* resembles in this respect the seal described by Turner (1875). The thickness of the placental labyrinth is also variable and a number of grooves on the placental surface are notable. The margins of the placenta are thin or moderately thick. The inner face of the placental belt is white in fixed material, on account of the allantochorion stretching under it. The allantochorion extends over the non-placental surface also, where it separates from the overlying residual tissue of the wall of the uterus. The blood vessels running on the foetal surface of the placenta are large, numerous and extend over the non-placental area also. The marginal haematomes are indistinct in naked-eye examination and no particular colour is noticeable in this part as it is in the case of cat and dog.

PLACENTAL LABYRINTH.—This region is prominent on account of its great thickness (6 mm.) and the parallel arrangement of villi and lamellae (Fig. 7). These run mostly vertically in the thickness of the placenta but a number of these is also found running obliquely and horizontally. In each villus can be noticed the mesenchyme of stellate cells as stated above and foetal vessels of different calibers. In the lamellae are seen maternal vessels having endothelia of flattened cells, whose outlines are not made out (Fig. 8), but which possess the nuclei comparatively larger than those in the adjoining syncytiotrophoblast. The maternal vessels are relatively larger towards the foetal surface. The placenta is endotheliochorial according to Otto Grosser (1910), as the endothelium of the maternal vessels is preserved. Thus the nourishment to be transported from the maternal to the foetal vessels has to traverse through the following barriers: (1) maternal endothelium of flattened cells, (2) a layer of syncytiotrophoblast having a thick zone of cytoplasm and (3) foetal endothelium of moderately flattened cells. The presence of 'giant cells' in the region of placental labyrinths in cat and dog has been recorded by previous investigators and the placenta of tiger differs in the absence of these. The red blood corpuscles in foetal and maternal vessels are enucleated.

TRANSITION ZONE.—The thickness of this zone is 0·4 mm. and at most places it contacts the layer of muscularis on the outer side. The tips of the villi are not as broad as in *Herpestes*. The structure of the syncytiotrophoblast on the top of the villi is variable. At places the nuclei are in several rows and the cytoplasm is granular and at other places the syncytiotrophoblast is very thin having elongated nuclei. Much of the tissue in this zone is shrunk partly on account of its nature and partly on account of the fixative used. However, masses of degenerated nuclei and cytoplasm, extravasated blood and dilated maternal vessels are very distinct.

DEEP GLANDULAR ZONE.—This zone is absent at most places and wherever it can be made out it is hardly 0·3 mm. in thickness. The basal portions of the circumferentially compressed uterine glands can be seen here and there only. The uterine mucosa on the outer side of the glands is difficult to be found out.

HAEMATOMES—These are present at the margins of the



FIGS. 8—10. Placentation in *Panthera tigris tigris*.
8. Section of the placental labyrinth ;
9. Section of extra-placental allantochorion
10. Section of a part of the gestation sac.

placental belt as are found in *Felis, Canis* and *Herpestes.* However, in *Panthera tigris* these are very small and dried up mostly. These are made of allantochorion and possess fine blood vessels.

MUSCULARIS.—This layer is 0·4 mms and more in thickness and its division into circular and longitudinal muscles is almost equal. The muscle fibres are greatly elongated and have wide intercellular spaces between them. The muscularis is richly vascularised.

EXTRA-PLACENTAL REGION.—The trophoblast is disorganised and only at places its cylindrical and cuboidal nature can be made out. The mesenchyme in this part consists of compact stellate cells and foetal vessels which possess flattened endothelium.

Panthera tigris greatly resembles *Felis* in its placentation. *Panthera tigris* possesses a zonary placenta, with more or less vertically disposed villi and lamellae, maternal vessels dilated more in the proximity of the foetal surface of the placenta, haematomes poorly developed and endotheliochorial nature of the placenta. The differences in the placental morphology of *Panthera tigris* and *Felis* are also striking. In *Panthera tigris* the zonary belt is not equatorial but in the proximity of the poles ; the width of the placental belt is not uniform but unequal. The 'giant-cells' are absent and the deep glandular zone is negligible. Thus genus *Panthera* is clearly demarcated from the genus *Felis* in so far as the placental morphology of the two is concerned.

SUMMARY

The placenta is zonary. Its orientation is in the proximity of one of the poles of the gestation sac. The width of the placental belt is unequal as in seal, a feature not so far recorded in the family Felidae. The placenta is lamelliform and the arrangement of the villi and lamellae mostly vertical to the placental surface and parallel to one another. The type of the placenta is endotheliochorial. The 'giant-cells' are absent. The zone of transition extends mostly up to the muscularis and the layer of deep glandular zone is almost obliterated. The marginal

haematomes are poorly developed and have ceased to function at most places.

REFERENCES

1. Balfour, F. M. 1881. Comparative Embryology.
2. Bonnet, R. 1920. Entwicklungsgeschichte.
3. Duval, M. 1893. Le placenta des Carnassiers.
4. Jenkinson, J. W. 1913. Vertebrate embryology.
5. Otto Grosser. 1909. Vergleichende Anatomie und Entwicklungsgeschichte der Eihdute und der Placenta.
6. Pocock, R. I. 1939. Fauna of British India. Mammalia Vol. 1.
7. Rau, A. S. 1925. Contribution to our knowledge of the structure of the placenta of Mustelidae, Ursidae and Sciuridae. *Proc. zool. Soc.*, 3.
8. Robinson, A. 1903. Lectures on the early stages in the development of Ova and on the formation of Placenta in different groups of Mammals (Lecture II) *Anat. Physiol.*, 38.

INDEX TO LETTERING

AC	Allantochorion
AM	Amnion
AV	Allantoic vesicle
BV	Blood vessel
D	Detritus
E	Embryo
EC	Exocoelome
E.C	Ectoderm cells
EN	Endoderm
F.T	Fallopian tube
F.V	Foetal vessel
GS	Gestation sac
H	Haematome
M	Mesometrium
MS	Muscularis
ME	Mesenchyme
N	Nucleus
NPA	Extra-placental region
PB	Placental belt
PL	Placental lapyrinth
R	Right side
SP	Splanchnopleur
TV	Top of a villous
TZ	Zone of transition
U	Umbilical cord
V	Villous
VV	Vitelline vessel
YS	Yolk sac

SOME GALL-MIDGES (ITONIDIDAE: DIPTERA) ASSOCIATED WITH *MANGIFERA INDICA* LINN. IN INDIA AND THEIR GALLS*

By M. S. Mani, M. A., D. Sc., F. Z. S. I., F. R. E. S., F. L. S., *Professor of Zoology & Entomology, School of Entomology, St. John's College, Agra.*

A number of gall-midges cause galls on *Mangifera indica* Linn. in various parts of the world. Sixteen different midge galls, from thirteen of which the midges are known, have so far been described from the world. These include, in addition to the ten species occurring within the limits of our faunal region, three New World species viz., *Asynapta mangiferae* Felt[1] from mango bark and *Erosomyia mangiferae* Felt[2] from mango leaf blister-gall from the West Indies and *Mangodiplosis mangiferae* Tavares[3] causing gall on the inflorescence axis of mango in Brazil. The midges from three galls occurring in India have not so far been reared. Most of the Indian species cause galls on leaves, two species on branches and one in flowers. The synoptic key at the end of this paper should be of help in the recognition of these galls.

Tribe DASYNEURINI

Dasyneura mangiferae Felt

1927. *Dasyneura mangiferae*, Felt, *Mem. Dept. Agric. India*, Ent. Ser., **10** : 1.
1934. *Dasyneura mangiferae*, Mani, *Rec. Indian Mus.*, **36** : 404.
1948. *Dasyneura mangiferae*, Mani, *J. Roy. Asiatic Soc. Bengal*, (Sci.) **14** : 91.

This species causes gall No. 195 on the flower buds in South India. The species was originally described from female specimens reared from flower galls at Coimbatore. In 1939 I received specimens from Cochin, where it was reported to have galled over 70% of the mango flowers during 1938-1939. Barnes[4] refers to specimens in his collection labelled as having been reared from flower galls of mango in Malaya. The species has so far not been found in north India.

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*Contribution No. 24 from the School of Entomology, St. John's College, Agra.

[1] Felt, E.P. 1909. *Ent. News*, **20** : 299.

[2] Felt, E.P. 1911. *J.N.Y. ent. Soc.*, **19** : 31.

[3] Tavares, J.S. 1918. *Broteria* (Zool.), **16** : 21.

[4] Barnes, H.F. 1948. Gall-midges of economic importance. Crossby, Lockwood & Sons. London, p. 94.

The female lays a single egg in each flower bud, which develops into a small, pointed, cone-shaped gall. Pupation also takes place within the gall.

Tribe OLIGOTROPHINI

Alassomyia tenuispatha (Kieffer)

1908. *Oligotrophus tenuispatha*, Kieffer, *Marcellia*, **7** : 150 (Larvae only).
1945. *Alassomyia tenuispatha*, Nayar, *Indian J. Ent.*, **6** : 71.
1948. *Alassomyia tenuispatha*, Mani, *J. Roy. Asiatic Soc. Bengal*, (Sci.), **14** : 91, 136.

This species gives rise to gall No. 190 on the leaf. The species was originally described by Kieffer (*loc. cit.*) from the galls and larvae only and the adult midge was unknown until in 1945 K.K. Nayar reared a few specimens. This made possible the correct generic placement of the midge.

This species is widely distributed in India. Its gall is among the largest that form on leaves and often measures from 2·5 mm. to 3 mm. in diameter. Globose, solid, epiphyllous or hypophyllous but visible on one side of the leaf only, unilocular, cystiferous, indehiscent, sessile, solitary, or in close groups of 3-4, also sometimes agglomerate, slaty-brown to black and sometimes when old very dark brown, with reticulate bark. Pupation in the gall.

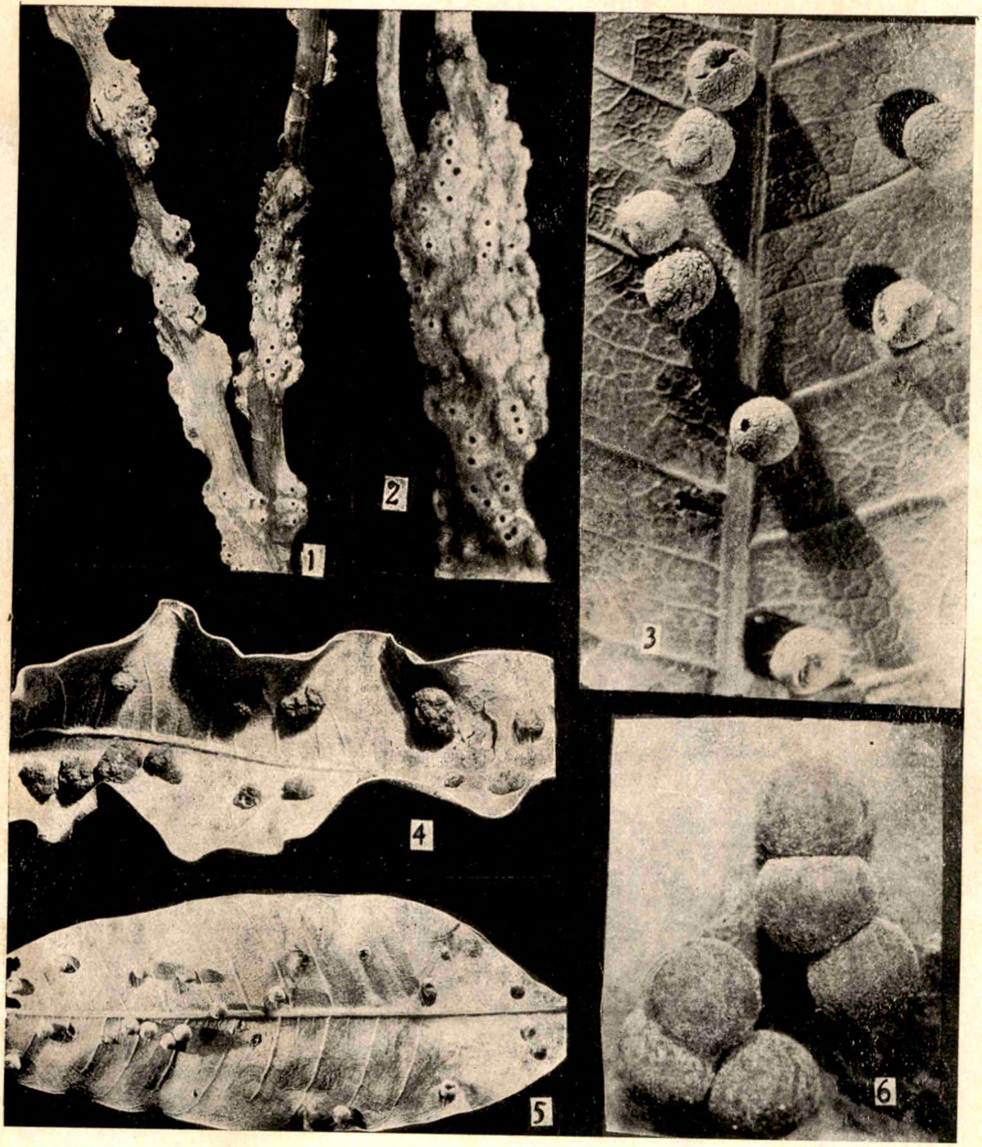
Oligotrophus mangiferae Kieffer

1908. *Oligotrophus mangiferae*, Kieffer, *Marcellia*, **7** : 15.

This species has remained unrecognized since Kieffer described the galls and the larvae only.

Rhabdophaga mangiferae Mani

1938. *Rhabdophaga mangiferae*, Mani, *Rec. Indian Mus.*, **49** : 331.
1948. *Rhabdophaga mangiferae*, Mani, *J. Roy. Asiatic Soc. Bengal*, (Sci.), **14** : 92.



1 & 2. Gall No. 197 by *Rhabdophaga mangiferae* Mani ; 3 & 5. Gall No. 325 by *Amradiplosis amraemyia.* (Rao) ; 4 & 6. Gall No. 190 by *Alassomyia tenuispatha* (Kieff.) (*Photos by author with Leica-Hektor* 13·5).

This species causes gall No. 197 on shoot of mango in South India. The species was originally described from specimens received from Cochin. Recently Dr. S. N. Rao, one of my pupils, brought several examples of the gall from Guntur on the east coast.

The gall is irregular, cortical, often extensive, globose or fusiform, multilocular, solid, tuberculated, indehiscent swellings on tender branches, with numerous exit holes covering the entire length. This is probably the same gall which was described by Docters van Leeuwen-Reijnvaan[1] as a lenticular cortical swelling of branches from Java.

The species appears to be fairly widely distributed in South India. The infestation is relatively very heavy in certain localities, especially during spring. Nearly every newly formed twig bears a dozen or more of the characteristic swellings, while others closeby remain entirely free. The galls attain their maximum size in April and the emergence of the adult flies continues from May to July. There is apparently a single annual generation. Pupation takes place in the gall.

Tribe BIFILINI

Genus **Amradiplosis** Mani

1947. *Amradiplosis*, Mani, *Bull. ent. Res. London*, **38** (3) : 441.
1950. *Amraemyia*, Rao, *Indian J. Ent.*, **10** (1) : 33. (New syn.).

This genus was erected by me for a unique midge which produces the remarkable echinate gall No. 196 on leaves, resembling a miniature sea-urchin. A careful comparison of the type and other material has convinced me that the recently erected *Amraemyia* Rao is identical with *Amradiplosis* Mani. The four species described by Rao under his *Amraemyia* are therefore transferred to the genus *Amradiplosis* Mani.

Key to species

1. Empodium shorter than claw 2
Empodium equal to or longer than claw 3

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[1] Docters van Leeuwen-Reijnvaan, J. & W. 1914. *Bull. Jardin Bot. Buitenzorg*, (2) **15** : 43, No. 416 a ; Houard, C. 1922. Les Zoocecidies des plantes d' Afrique, d' Asie et d' Oceanie, **1** : 466, No. 1713.

2. Antennae longer than body ; second palpal segment in male equal to the third ; third palpal segment in female one and a half times the second ... ... *Amradiplosis brunneigallicola* (Rao)

   Antennae longer than body ; third palpal segment in male longer than second ; in female one and one-third the second *Amradiplosis keshopurensis* (Rao)

   Antennae about half the length of body ; second and third palpal segments in male subequal ; third palpal segment in female nearly twice the second ... *Amradiplosis amraemyia* (Rao)

3. Empodium equal to claw ; second and third palpal segments in the female subequal ; third palpal segment in male shorter than the second ... ... *Amradiplosis viridigallicola* (Rao)

   Empodium longer than claw ; third palpal segment in the female nearly twice the second ; in male about one and one-fifth to slightly less than twice the second ... *Amradiplosis echinogalliperda* Mani

Amradiplosis echinogalliperda Mani

1947. *Amradiplosis echinogalliperda*, Mani, *Bull. ent. Res. London.*, **38** (3) : 443.
1948. *Amradiplosis echinogalliperda*, Mani, *J. Roy. Asiatic Soc. Bengal*, (Sci.) **14** : 92.

This species causes the echinate gall No. 196 on leaves. It is widely distributed in Bengal, Bihar and Uttar Pradesh, especially in the northern parts.

The gall of this species was first described by me[1] in 1935 from specimens collected at Calcutta. Epiphyllous, solid, globose, regular, free, solitary, sessile, sometimes conglomerate, uni- or bilocular, indehiscent, cystiferous, sometimes green when young, dark reddish brown to nearly reddish-black when old, with dense long, fleshy, spine-like emergences.

The adults emerge once in February-March and again in October. Some of the progeny of the flies emerging in March complete the life-cycle in October, but the others over-winter in the gall as larvae to complete the development in February next. This species has not so far been found in South India.

Amradiplosis viridigallicola (Rao)

1950. *Amraemyia viridigallicola*, Rao, *Indian J. Ent.*, **10** : 34.

This species gives rise to gall No. 324 on leaves. Usually the gall is epiphyllous, glabrous, solid, globose, sessile, free,

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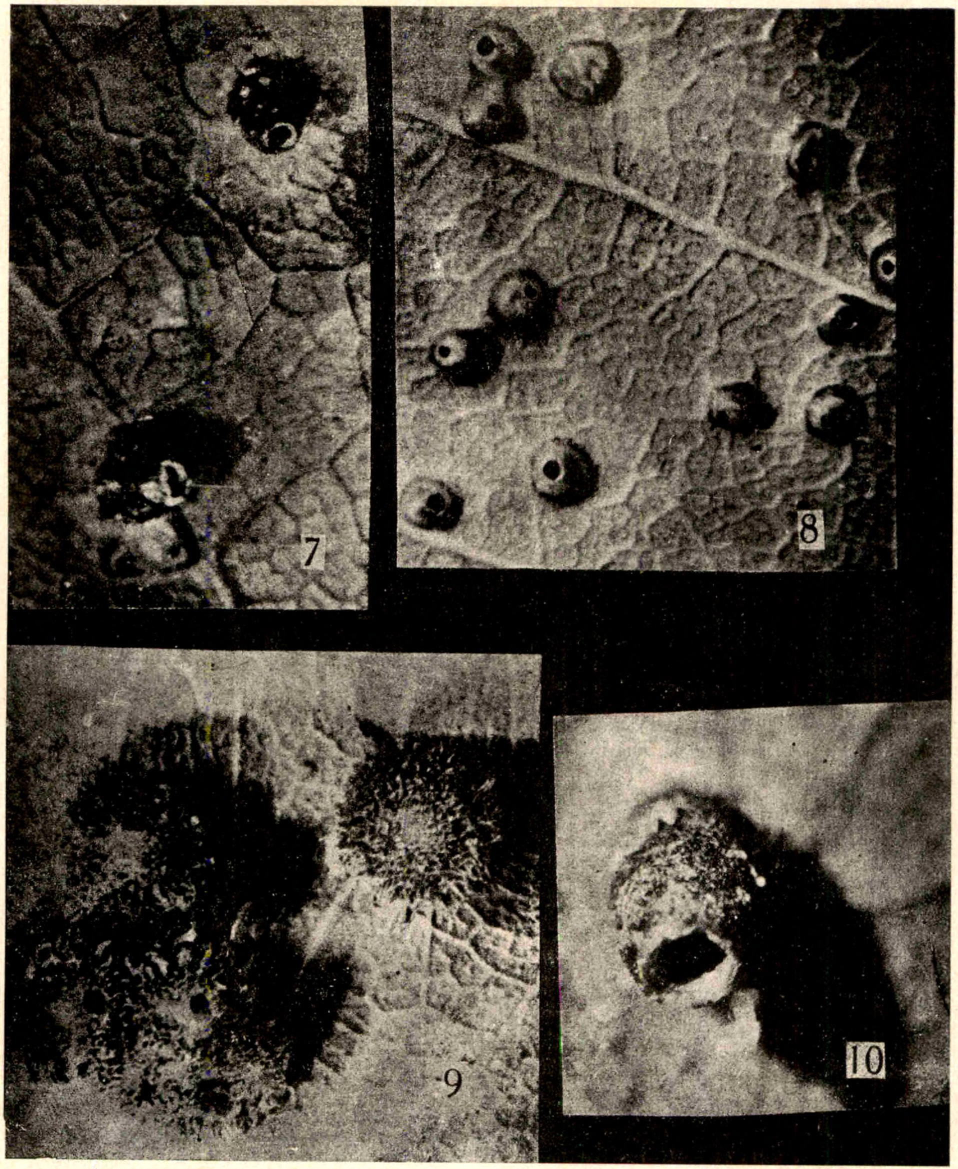
[1] Mani, M.S. 1935. *Rec. Indian Mus.*, **37** : 446.



7 & 10. Gall No. 191 by an unknown midge ; 8. Gall No. 192 by *Procontarinia matteiana* Kieff. *et.* Cecc. ; 9. Gall No. 196 by *Amradiplosis echinogalliperda* Mani. (*Photos by author with Leica-Hektor* 13·5).

solitary, unilocular, indehiscent, simple and about 1 mm. in diameter, yellowish-green and shiny.

The species appears to be extremely common in several parts of Madras, Bengal, Bihar and Uttar Pradesh.

There is a single annual generation. The adults usually emerge in February-March or early April in certain localities. The galls mature by January next. The total pre-imaginal period is about 250 days, but the adult is relatively short-lived. The incubation period of the egg appears to be rather prolonged over 4 or 5 months.

Amradiplosis amraemyia (Rao)

1950. *Amraemyia amraemyia*, Rao, *Indian J. Ent.*, **10** : 37.

The species gives rise to gall No. 325 on leaves. The gall differs from that of the foregoing species in its larger size. Epiphyllous, solid, globose, solitary, sessile, simple, free, unilocular, indehiscent and slaty-grey. The emergence of the adult occurs about April and the galls mature in next February.

Amradiplosis brunneigallicola (Rao)

1950. *Amraemyia brunneigallicola*, Rao, *Indian J. Ent.*, **10** : 39.

This species causes gall No. 326. This gall differs from those of the other species in being small, brown, globose, epiphyllous, solitary, simple, free, solid, sessile, shiny, unilocular, indehiscent and with a shiny-brown cap-like operculum over the future exit hole on the summit of the gall.

The adult midge emerges in April and the galls mature in July-August next. The larvae however over-winter within the mature gall before pupating in March next. The total life-cycle is about one year and the pre-imaginal period extends to about 350 days.

Amradiplosis keshopurensis (Rao)

1952. *Amraemyia keshopurensis*, Rao, *Proc. R. ent. Soc. London*, (B) **21** (3-4) : 52.

This species was reared from Gall No. 332 on leaf. The gall is yellow-coloured, globose, epiphyllous and hardly 1 mm. in diameter. The midges emerge in November.

Indodiplosis mangiferae Felt

1916. *Indodiplosis mangiferae*, Felt, *Canadian Ent.*, **48** : 403.

This species was described from specimens from "leaf galls" on mango at Pusa. The gall has not so far been recognized.

Procontarinia matteiana Kieffer & Cecconi

1906. *Procontarinia matteiana*, Kieffer & Cecconi, *Marcellia*, **5** : 135-136, fig. 1-3.
1906. *Procontarinia matteiana*, Stefani-Perez, *Marcellia*, **5** : 165.
1934. *Procontarinia matteiana*, Mani, *Rec. Indian Mus.*, **36** : 420.
1943. *Procontarinia matteiana*, Mani, *Indian J. Ent.*, **5** : 159.

This species gives rise to gall No. 192 on leaf and is perhaps the commonest gall midge associated with mango in India. Nearly every square millimetre of every leaf is covered by its characteristic flat, discoid, circular, yellow to pink-coloured, solid, pustuloid galls, about 2 mm. in diameter, often over 300 per leaf, and visible on both surfaces of the leaf.

The species was first described from specimens which emerged from the galls on mango plants just imported into Sicily from India. The species also occurs in Java, Mauritius and South Africa.

The adults from the over-wintering larvae (in the gall) emerge about the middle of March and oviposit on the tender and newly forming leaves in early spring. Oviposition may continue to the end of May. The incubation period of the eggs extends from 3 to 4 days. The larval period ranges from 120 to 386 days. The pupal period lasts from 7 to 10 days during late February or early March and pupation takes place within the gall. The adult midges usually emerge in the morning.

There are possibly 3 or 4 annual generations that freely overlap : the first generation extends from March to July, the second from July to nearly the middle of October and the third from October to March. The adults of the first generation do not all emerge at one time in July, but continue to do so in 3 or 4 successive batches in July, October and March along with the adults of the second and third generations. The adults of the second generation emerge likewise partly in October, a second batch in March and a third batch in July next. The third generation flies emerge largely in March but a small number in July next. The total life-cycle can be as short as two and a half months or as prolonged as one whole year. The larvae are frequently heavily parasitized by a platygasterid.

The proportion of males is relatively high in the adults emerging in March-April and comparatively low in the July and October families. Parthenogenesis no doubt is interposed in some of the families.

GALLS FROM WHICH THE MIDGES HAVE NOT YET BEEN REARED

Gall No. 191. Tiny, cylindrical, epiphyllous, rarely hypophyllous, often obtusely conical, solid, glabrous, brown to dark reddish-brown, unilocular, indehiscent, sessile, solitary, free, simple, about 1 mm. thick basally and 1—2·5 mm. high, apically with a cap-like operculum that falls off, leaving a circular large hole in old galls ; on the opposite side of the leaf the site of the gall is marked by an obscure biconvex, brownish pustule.

This gall was described by Kieffer[1] from India and is also known to occur in Java, Sumatra and Sebesi Islands.[2]

Gall No. 194. Circular, biconvex, unilocular pustules, visible on both sides of the leaf, 1·5—3 mm. in diameter, glabrous, pink when old, with exit hole above or below. This gall was originally described by Kieffer (*loc. cit.*) from India and is likewise known to occur in Java.[3]

Key to the midge-galls on mango

1.	Gall on branches	2
	Gall on leaves or flowers, not on branches	3
2.	Fusiform, unilateral swellings of twigs caused by *Oligotrophus mangiferae* Kieff.	Gall No. 189

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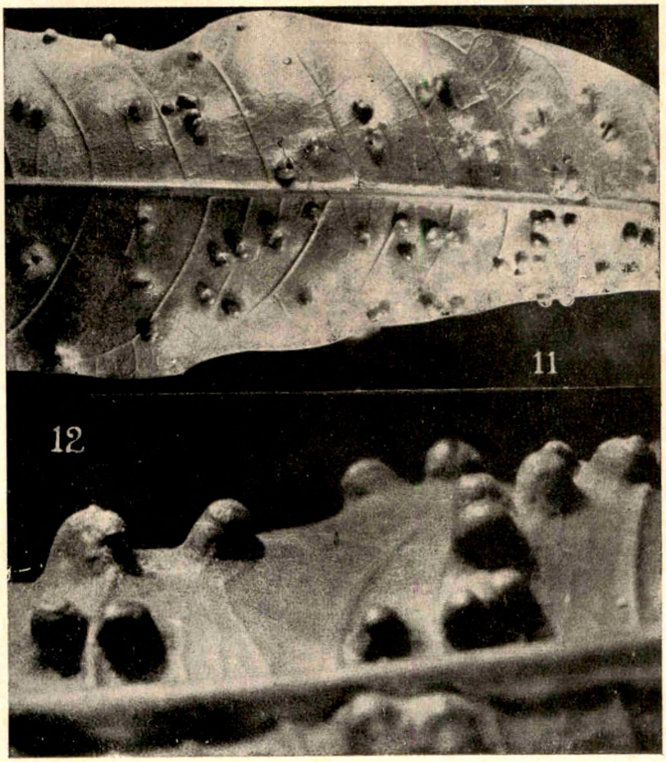
[1] Kieffer, J. J. 1908. *Marcellia*, **7** : 151.

[2] Houard, C. 1922. Les Zoocecidies des plantes d' Afrique, d' Asie et d' Oceanie, **7** : 466, N . 1716 ; Docters van Leeuwen-Reijnvaan, J. & W. Zoocecidia of Netherlands East-Indies, Buitenzorg, p. 324, fig. 578 ; Mani, M. S. 1948. *J. Roy. Asiatic Soc. Bengal*, (Sci.), **14** : 136.

[3] Mani, M. S. *op. cit.*, p. 137.

	Irregular, cortical, extensive swellings of twigs caused by *Rhabdophaga mangiferae* Mani	Gall No. 197
3.	Gall in flowers caused by *Dasyneura mangiferae* Felt ...	Gall No. 195
	Gall on leaves	... 4
4.	Gall visible on both sides of the leaf	... 5
	Gall epiphyllous or hypophyllous, but not equally developed on both sides of leaf	... 6
5.	Depressed, circular, discoid pustules by *Procontarinia matteiana* Kieff. & Cecc.	Gall No. 192
	Biconvex pustules by unknown midge	Gall No. 194
6.	Obtuse-conical or subcylindrical, usually epiphyllous ...	Gall No. 191
	Hemispherical, epiphyllous green galls by unknown midge ...	Gall No. 406
	Globose or pill-shaped galls	... 7
7.	Large galls, with fleshy emergences, resembling a miniature sea-urchin, caused by *Amradiplosis echinogalliperda* Mani	Gall No. 196
	Never with fleshy or other emergences, but smooth, at the most with the bark reticulated when mature	... 8
8.	Large, often over 3 mm. in diameter, dark slate-coloured or very dark brown, usually epiphyllous, caused by *Alassomyia tenuispatha* (Kieff.)	Gall No. 190
	Medium-sized or small galls, never more than 2 mm. in diameter	... 9
9.	Small, green galls by *Amradiplosis viridigallicola* (Rao) ...	Gall No. 324
	Small, pale yellow galls by *Amradiplosis keshopurensis* (Rao) ...	Gall No. 332
	Medium-sized galls, not green when mature	... 10
10.	Brown gall by *Amradiplosis brunneigallicola* (Rao) ...	Gall No. 326
	Slate-grey gall by *Amradiplosis amraemyia* (Rao)	Gall No. 325



11 & 12. Gall No. 406 by an unknown midge. (*Photos by author with Leica-Hektor* 13·5).

FOUR NEW SPECIES OF CHALCIDOIDEA (*PARASITIC HYMENOPTERA*) FROM INDIA*

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The type specimens of the new forms described in this paper belong to the collections of the Forest Entomologist, Forest Research Institute, Dehra Dun. The author's cordial thanks are due to the Forest Entomologist for lending this material and to Dr. M. S. Mani, Professor of Zoology and Entomology, School of Entomology, St. John's College, Agra, for facilities of work and guidance.

Tetrastichus plecopterae, sp. nov.

Male. Length : 1·75 mm. Colour : Black, with metallic green iridescence. *Head* viewed from above transverse, nearly dumbell-shaped, distinctly wider than thorax, width slightly over four times the length ; vertex narrow ; ocelli reddish-brown, arranged in a curved line ; interocellar space slightly less than twice the ocellocular, which is somewhat more than the front ocellar ; postocellar space very narrow ; viewed in front (Fig. 9) nearly triangular, length nearly three-fourths the width ; interorbital space three-fifths the width ; gena slightly less than two-thirds the orbit ; eyes large, brown, bare ; mandible dark reddish-brown *Antennae* (Fig. 10) clavate, light brown except for the black club, inserted below the middle of the face on an imaginary line joining the lower orbital borders ; 10-segmented ; scape, pedicel, one ring joint, four flagellar segments and triarticulate club ; scape nearly reaching the front ocellus, broadened in the middle, two and two-thirds the pedicel ; pedicel vase-shaped, slightly longer than first flagellar segment, but equal to the ring-joint and first flagellar combined ; flagellar segments gradually become shorter but thicker, the last one being nearly spherical ; club much thicker than the rest of antenna, slightly

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shorter than the last two flagellar segments combined; second segment the longest and nearly one and a half times the first, the terminal segment two-thirds the second, conical and apically with a nipple-like process. *Thorax*: Pronotum short, faintly transversely striate and shagreened, anteriorly indistinctly narrowing into a neck: mesonotum broader than pronotum with a dull, metallic bluish-green lustre, with a median longitudinal foveae distinct; parapsidal furrows deep, distinct and complete; scutellum as long as mesothorax, convex, with two distinct longitudinal furrows dividing it into three well defined areas, median area broader than the lateral; metanotum short with two broad lateral foveae; propodeum large, distinctly marked off from the metanotum by a carina, with median and lateral longitudinal carinae, basal carina also present; shallowly rugoso-punctate; mesopleura impressed, an obscure carina separating it into two, transversely striate; tegulae reddish-brown; metapleurae somoth and shiny. *Legs* light brown, except for the darker coxae; hind coxa long, nearly thrice the trochanter, femur twice the coxa; tibia nearly one and one-third the femur; metathorax nearly one-seventh the tibia and one-third the rest of the tarsi combined; tibial spur four-fifths the metatarsus (Fig. 8). *Fore wing* (Fig. 6, 7) sparsely pubescent; stigma and veins light brown; length two and one-third the breadth; submarginal vein very slightly longer than marginal or nearly equal; postmarginal absent; marginal three and a half times the stigmal with eight long bristles. *Abdomen* broad, dorsoventrally flattened; the first three tergites large, rest smaller, last conical; male genitalia exserted.

Holotype male dissected and mounted on slide No. $\frac{616}{C}$, *paratypes* many males on pins: labelled Ex. No. 948, parasite of *Plecoptera reflexa* larvae, *Dalbergia sissoo* Entomological Survey 1939, 16-ix-1939, coll. R.N. Mathur, New Forests, Dehra Dun.

This species is apparently similar to *Tetrastichus ayyari* Rohwer, but is distinguished from it by the distinctly three-jointed club; funicular segments gradually increasing in size; propodeum shallowly rugoso-punctate, with a distinct median longitudinal carina and two lateral carinae.

Aprostocetus punjabensis, sp. nov.

Female. Length: 1·75—2·0 mm. Colour: Metallic iridescent blue and green. *Head* viewed from above transverse, width slightly less than three times the length; vertex narrowed, faintly punctate; ocelli arranged in a broad triangle; interocellar distance three and a half times the ocellocular and slightly less than three times the front ocellar; ocellocular three-fifths the front ocellar; interorbital distance slightly more than half the width of the head; viewed in front mouth truncate, length three-fourths the breadth; clypeus short, but broad; face moderately convex, gena prominent, slightly less than two-thirds the length of eye; inner orbital borders parallel. *Antennae* (Fig. 16) inserted in the middle of face just above an imaginary line joining the lower orbital

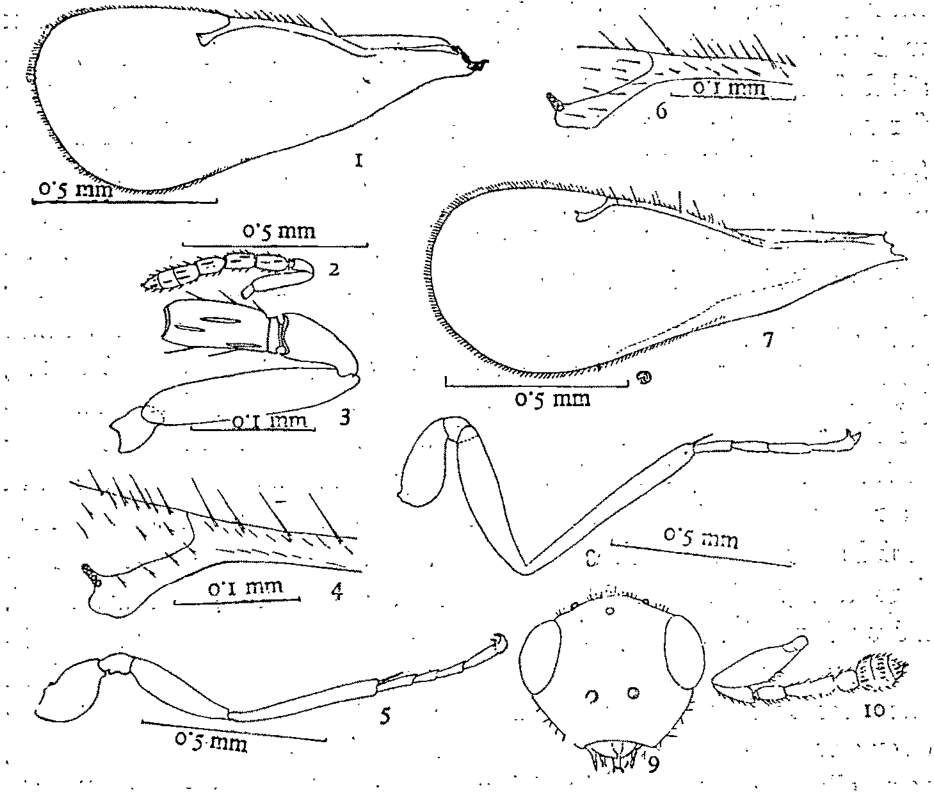

FIG. 1-5. *Aprostocetus nowsherensis*, sp. nov. female 1. Fore wing, 2. antenna, 3. basal segments of antenna enlarged, 4. stigma of fore wing enlarged, 5. hind leg.

FIG. 6-10. *Tetrastichus plecopterae*, sp. nov. male 6. stigma of fore wing enlarged, 7. fore wing; 8. hind leg, 9. head viewed in front, 10. antenna.

borders of eyes; 9-segmented; scape, pedicel, one ring joint, three-segmented flagellum and triarticulate club; scape long, yellowish brown; rest of antenna brown; scape slightly less than three times the pedicel; pedicel five times the ring joint; first flagellar segment very slightly more than half the scape and slightly longer than the rest of the flagellar segments; second and third flagellar segments together very slightly shorter than club, which latter is equal to the scape; the three segments of the club gradually become shorter; terminal segment with a nipple-like process at the tip. *Thorax*: prothorax metallic green with a bluish tinge, narrower in front, rugosopunctate; mesothorax metallic blue with a greenish tinge, wider than prothorax; parapsidal furrows distinct, deep and complete, median longitudinal furrow present; scutellum convex with two parallel longitudinal grooves, metallic blue with a greenish-yellow tinge, coriaciously striate; metathorax short, metallic blue; propodeum slightly convex, with a median longitudinal carina, which diverge posteriorly enclosing a triangular area; with fine leathery sculpture; mesopleura with a transverse fovea, anteriorly nearly smooth and shiny, posteriorly roughly sculptured. *Legs* (Fig. 18) except coxae, which have the same colour as the rest of the body, and brownish-black trochanters and femur, yellowish-brown; hind coxa nearly twice the trochanters; femur slightly over twice the coxae and four times the trochanters; tibia one and one-fifth the femur; tibial spur one-seventh the tibia and two-thirds the metatarsus; metatarsus slightly more than half the rest of tarsi; claw simple. *Fore wing* (Fig. 13, 14) length two and one-third the breadth; submarginal very slightly longer than the marginal; stigmal one-third the marginal; post marginal absent. *Abdomen* sessile; slightly over one and a half times the thorax; dorso-ventrally depressed, metallic green with blue iridescence; ovipositor very slightly exserted.

Holotype female dissected and mounted on slide No. $\frac{630}{\mathrm{C}}$; *paratypes* many females on pins, labelled RRD-313, *Dalbergia sissoo* Entomological Survey 1938-Expt. 66, 18-iv-1938, Terah Plantation, Punjab.

This species differs from *Aprostocetus israeli* Mani and

Kurian* in having the interocellar space three and a half times the ocellocular ; ocellocular only four-fifths the front ocellar ; antennae inserted at the middle of face, just above an imaginary line joining the lower orbital borders ; first flagellar very slightly more than half the scape.

Aprostocetus coorgensis, sp. nov.

Female. Length : 2—2·3 mm. Colour : Brilliant metallic blue with a greenish or violet tinge. *Head* viewed from above transverse, nearly dumb-bell-shaped, two and three-fourths as broad as long ; vertex narrow, roughly rugosopunctate, sparsely pubescent ; ocelli reddish-brown, arranged in a broad triangle ; interocellar space two and one-fourth the ocellocular, which is very nearly equal to the front ocellar ; interorbital space very slightly more than half the width of the head ; eyes brown, bare ; viewed in front somewhat triangular, length slightly more than three-fourths the breadth ; interorbital space four-sevenths the breadth of the head ; gena three-fifths the length of the eye, a groove traversing the gena longitudinally, extending from the lower border of the eye to the sides of the clypeus. *Antennae* (Fig. 15) inserted slightly below the middle of the face, just above an imaginary line joining the lower orbital borders ; antennal foveae run up to nearly the front ocellus ; antenna dark brown, scape paler ; 9-segmented ; scape, pedicel, single ring-joint, 3-segmented flagellum and triarticulate club ; scape long, cylindrical, three times the pedicel ; pedicel seven times the ring-joint ; first flagellar segment three-fifths the scape ; the rest of the flagellar segments relatively shorter, third segment half the scape ; club slightly shorter than the last two flagellar segments combined, which are together equal to the scape ; flagellar segments gradually become shorter ; the last two being nearly equal ; terminal segment conical with a short nipple-like process, at the base of which are two other short spiny processes. *Thorax* : pronotum brilliant metallic blue, leathery punctate ; mesothorax not so brilliant, nearly of the same colour, but with a greenish tinge ; parapsidal furrows deep and complete ; median longitudinal groove present ;

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*Description in press in the *Indian Journal of Entomology.*

scutellum convex, rounded posteriorly, with two lateral furrows dividing it into three equal areas ; colour same as mesothorax except having a little more of the greenish tinge ; finely longitudinally coriaciously striate ; metathorax narrow ; propodeum carinate medially and laterally, with distinct rugoso-punctae. *Legs* (Fig. 17) except coxae and part of femur yellowish-brown ; coxa concolouress with thorax ; hind coxa two and one-third as long as the trochanter, which is one-fourth the femur; femur three-

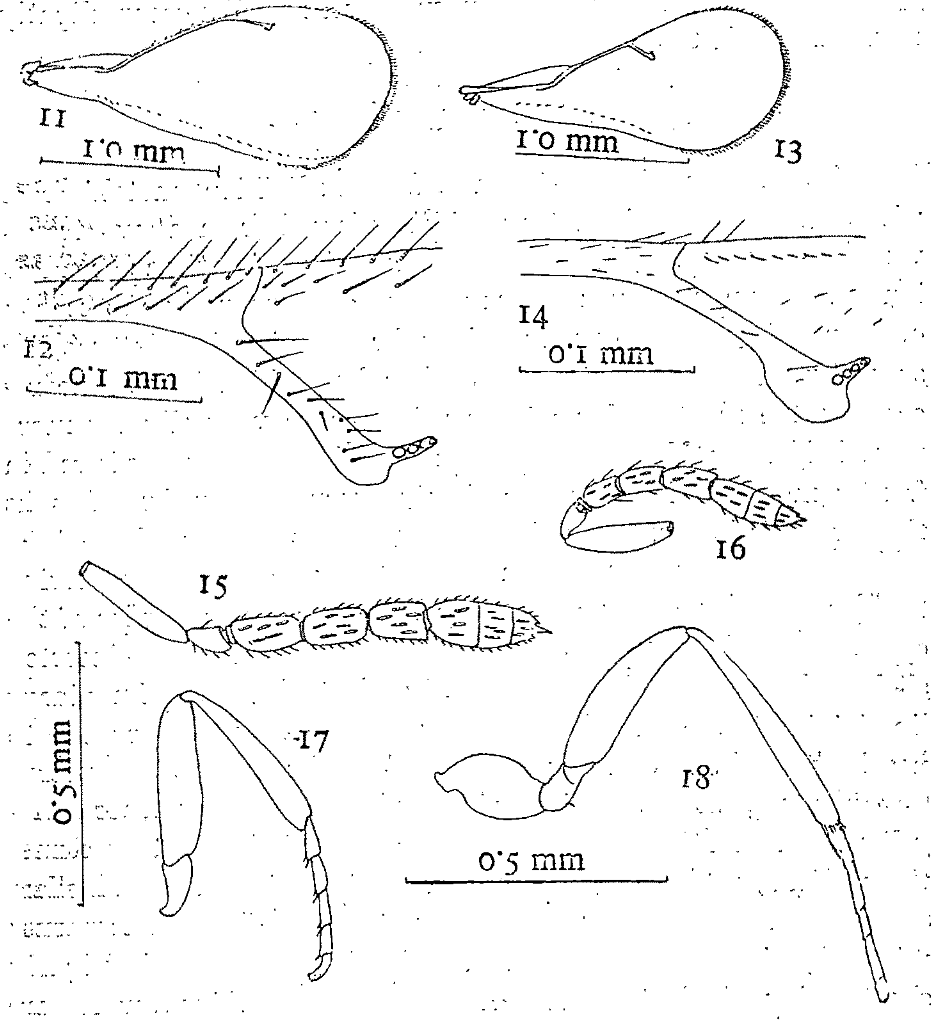

FIG. 11, 12, 15 and 17. *Aprostocetus coorgensis*, sp. nov. female 11. fore wing, 12. stigma of fore wing enlarged, 15. antenna, 17. hind leg.

FIG. 13, 14, 16 and 18. *Aprostocetus punjabensis*, sp. nov. female 13. fore wing, 14. stigma of fore wing enlarged, 16. antenna. 18. hind leg.

fourths the tibia ; tibial spur nearly one-sixth the tibia, which is equal to the metatarsus ; rest of tarsi thrice the metatarsus. *Fore wing* (Fig. 11, 12) length two and one-fourth the width ; submarginal slightly longer than the marginal which is slightly more than thrice the stigmal, postmarginal absent. *Abdomen*: sessile, nearly same colour as the thorax, dorso-ventrally depressed ; apex acuminate ; dorsally smooth and ventrally shagreened.

Holotype female dissected and mounted on slide No. $\frac{619}{\mathrm{C}}$; *paratypes* many females on pin, labelled RRD. 987, parasite of Pyralid larva parasitising *Grewia latifolia*, 2-x-1940, Coll : B. M. Bhatia, Tithimatti, S. Coorg.

This differs from the foregoing species in having the interocellar space two and one-fourth the ocellocular ; ocellocular nearly equal to the front ocellar ; antenna inserted slightly below the middle of face ; club slightly shorter than the last two flagellar segments combined.

Aprostocetus nowsherensis, sp. nov.

Female. Length : 1·5—2·0 mm. Colour : Brilliant metallic blue and green. *Head* metallic green ; viewed from above transverse ; 3 times as wide as long ; eyes blackish-brown, bare, large ; interorbital distance slightly less than two-thirds the width of the head ; ocelli reddish-brown, arranged in a curved line ; interocellar distance slightly more than the ocellocular, which is equal to the front ocellar ; vertex narrow, emarginate, rugosostriate, sparsely pubescent; viewed in front nearly round, eyes bulging; face excavate ; front ocellus in an excavation ; faintly rugosocarinate ; genal length slightly less than half the orbit. *Antenna* (Fig. 2, 3) inserted nearly in the middle of the face, just above an imaginary line joining the lower orbital borders ; brown except for yellowish-brown scape and pedicel ; scape nearly three times the pedicel, which is slightly over six times the ring-joint ; first flagellar segment nearly one and a half times the pedicel ; second very slightly longer than the first ; third slightly shorter than the second ; club triarticulate, segments gradually decreasing in length ; all three together nearly equal to the last two flagellar segments combined; apex of club with a short nipple-like process. *Thorax* metallic green, with a bluish-yellow tinge; prothorax short, rugosopunctate in the middle and rugosostriate at the sides; mesonotum concolouress with pronotum; rugosostriate with distinct and complete parapsidal furrows; lateral oblong foveae on the outside of the parapsidal furrows on each side; scutellum large with two lateral longitudinal parallel furrows, which slightly diverge anteriorly; sculpture same as that of mesonotum; axillae clearly well defined and separated from scutellum; metanotum short, narrowed at the sides, indistinctly rugosopunctate, propodeum rugosopunctate with a short median longitudinal carina, which bifurcates at the posterior end and runs transversely as very strongly defined basal carina; spiracles medium-sized, oval. *Legs* (Fig. 5) Coxa, trochanter and basal half of femur dark reddish-brown; rest of legs yellowish brown; hind coxa two and three-fourths the trochanters; femur one and one-third the coxa and trochanters combined; tibia nearly one and one-fourth the femur; tibial spur short, one-sixth the tibia; metatarsus slightly less than one-fifth the tibia and one-third the rest of the tarsi combined. *Wings* (Fig. 1, 4) sparsely pubescent; fore wing length somewhat more than twice the breadth; submarginal slightly more than marginal; postmarginal absent. *Abdomen* sessile; one and a half times the length of thorax; dorso-ventrally depressed, apex attenuate.

Holotype female dissected and mounted on slide No.$\frac{613}{C}$; *Paratypes* many females on pins labelled Expt. No. 168, RRD 327, *Dalbergia sissoo* Entomological Survey 1938 9-V-1938, Nowhsera, N.-W.F.P. (Now Pakistan).

This species differs from the foregoing two species in having the interocellar slightly more than thrice the ocellocular; first flagellar just half the scape and club equal to the last two flagellar segments combined.

DESCRIPTIONS OF FOUR NEW AND RECORD OF ONE KNOWN BETHYLOIDEA (*PARASITIC HYMENOPTERA*) FROM INDIA.*

By Chandy Kurian, M.Sc., F.R.E.S., *Research Associate, School of Entomology, St. John's College, Agra.*

The type specimens of the new forms described in this paper belong to the collection of the Division of Entomology, Indian Agricultural Research Institute and to Dr. M. S. Mani, Professor of Zoology and Entomology, St. John's College, Agra. The author is grateful to the Head of the Division of Entomology, Indian Agricultural Research Institute, for lending the material and to Dr. M. S. Mani, Professor of Zoology and Entomology, St. John's College, Agra, for facilities of work and guidance.

Goniozus nephoterycis, sp. nov.

Male. Length : 2.75 mm. Colour : dark reddish-brown to black. *Head* viewed from above length somewhat more than half the width ; interorbital distance very slightly over three-fourths the width ; vertex minutely leathery punctate, covered with few scattered setae ; convex, emarginate, but laterally faintly margined ; occiput excavate ; ocelli brown, arranged in a broad triangle ; interocellar distance three-fifths the ocellocular, which latter is thrice the front ocellar ; viewed in front nearly round, length somewhat less than the width ; interorbital distance somewhat more than three-fifths the width ; frons and face punctation same as in the vertex, sparsely but uniformly covered over by silvery-white pubescence ; length of eye four and a half times that of gena ; post orbital slightly less than half the length of eye ; eyes brownish-black, bare ; clypeus narrow, beak-like, with a carina running longitudinally from apex up to the level about one-third of the length of eye ; mandibles reddish-brown, with darker teeth ; viewed from side elongate-oval shaped ; temple narrow, one-third the

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breadth of eye. Antenna inserted above the mouth on either side of the clypeus, reddish-brown, with darker apex ; scape very slightly longer than twice the pedicel ; first flagellar segment very slightly shorter than the pedicel ; rest of the flagellar segments very gradually become longer and then shorter ; penultimate slightly shorter than the pedicel ; terminal a little less than one and a half times the penultimate and five-eighths the scape (Fig.3).

Thorax somewhat less than twice the breadth in the region of the tegulae ; dark reddish-brown ; prothorax divided into a narrow anterior region and a gradually broadening out posterior region by a carina, faintly leathery sculptured with a shagreened appearance, posterio-laterally extending as two distinct spinous processes one on either side ; mesothorax half the prothorax ; tegulae reddish-brown ; mesonotal punctation same as in the anterior region of the prothorax ; posteriorly nearly smooth and shiny ; scutellum flattened, roughly triangular, nearly the same length as the mesothorax, nearly smooth and shiny posteriorly, with a faint irregular leathery sculpture anteriorly ; metathorax one-third the scutellum ; punctation same as in the anterior area of the scutellum, propodeum nearly twice the size of scutellum, with a distinct leathery sculpture in the anterior horizontal part, which is differentiated from the posterior declivous area by a transverse carina ; anteriorly with a triangularly elevated area ; propodeal spiracles large, leniar. Legs dark reddish-brown, with lighter tarsi. Fore coxa slightly more than one and a half times the trochanter ; femur somewhat less than thrice the trochanter ; tibia three-fourths the femur ; tibial spur slightly less than one-third the tibia ; metatarsus three-fifths the tibia and slightly more than two-thirds the rest of tarsi combined (Fig. 4). Hind coxa two and one-fourth the trochanter ; femur four times the trochanter ; tibia slightly more than the femur ; tibial-spur one-fifth the tibia and two-fifths the metatarsus ; metatarsus half the tibia and very slightly less than three-fourths the rest of tarsi combined ; claw bifid (Figs. 5 and 6). Fore wing length slightly more than two and a half times the breadth ; radius very slightly more than one-sixth more the length of the wing ; basal stalk of basal slightly over one-fifth the radius ; anterior branch of basal slightly more than one and three-fifths the basal stalk and equal to the posterior ; transversal equal to the basal stalk of basal (Fig. 1 and 2).

Abdomen: Dark reddish-brown, slightly shorter than the throax ; only six tergites clearly visible, the first few larger and lighter in colour than the rest.

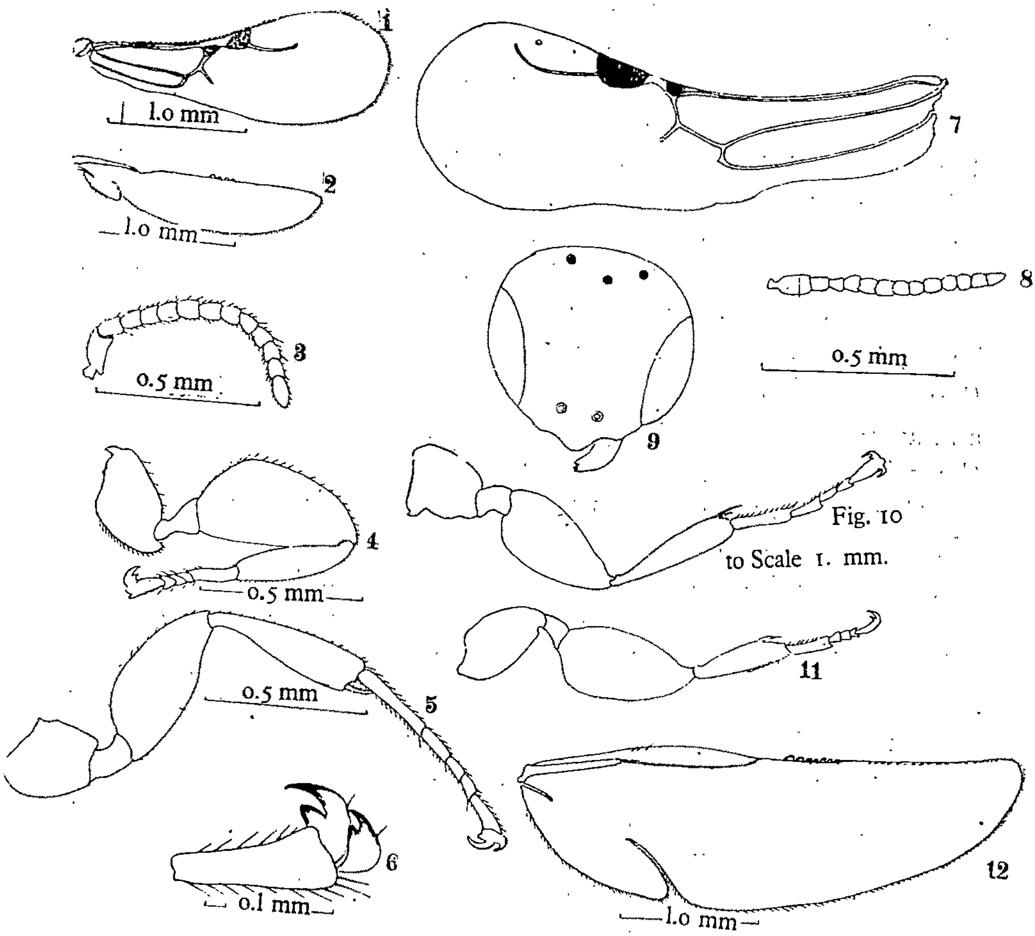

FIG. 1–6. *Goniozus nephoterycis*, sp. nov. Male. 1. fore wing, 2. hind wing, 3. antenna, 4. fore leg 5. hind leg, 6. claw of hind leg enlarged.

FIG. 7–11. *Goniozus morindae*, sp. nov. Male. 7. fore wing, 8. antenna, 9. head viewed in front, 10. hind leg, 11. fore leg.

FIG. 12. *Pristepyris agraensis*, sp. nov. Male. 12. hind wing.

Holotype: Male on pin No. $\frac{1}{B}$ partly dissected and mounted on slide No. $\frac{1}{B}$ labelled : parasite C. No. 2588, *Nephoteryx* larvae binding leaves of *Cassia fistula*, 9-11-1931, Pusa.

This differs from *Goniozus indicus* Ashmead to which it is related in having the frons and face distinctly shagreened ; post orbital slightly less than half the length of the eye ; legs dark reddish-brown and propodeal transverse carina being present.

Goniozus morindae, sp. nov.

Male. Length : 2.50 mm. Dark reddish-brown. *Head* viewed from above nearly rectangular, flat, finely shallowly leathery punctate, pubescent ; clypeus without distinct carina, mandibles reddish-brown. Eyes large and bare ; length two and a half times the post occipital region ; ocelli yellow, arranged in a broad triangle ; interocellar space nearly one and a half times the front ocellar and slightly more than three-fourths the ocellocular ; posterior ocelli placed in the margin of the vertex ; vertex indistinctly margined ; Viewed in front very slightly broader than long ; interorbital distance in the middle of the face slightly less than two-thirds the width of the head ; frons and face shagreened and sparsely pubescent (fig. 9). Viewed from side temple nearly half the width of eye ; gena very slightly less than one-fourth the length of eye. Eyes yellowish-brown ; post orbital distance somewhat less than half the length of eye. Antenna uniformly honey yellow, covered over by short pubescence, longer than the head ; inserted on the clypeus on either side ; scape thick, cylindrical, nearly as long as the pedicel and first flagellar combined ; first two flagellar segments serrate, rest of them gradually become moniliform ; terminal segment slightly longer than the penultimate (fig. 8) ; vertex indistinctly margined ; occiput excavate. *Thorax* Prothorax long, narrowed anteriorly, longer than mesothorax, shagreened, sparsely pubescent ; mesothorax short, transverse, smooth and shiny except for very shallow, scattered punctures, longer than scutellum ; parapsidal furrows indistinct ; scutellum slightly convex with two foveae anteriolaterally one on either side ; propodeum large, margined laterally and behind, leathery, with a smooth median triangular ares placed anteriorly continuing as a carina which runs down traversing the whole of the horizontal area of the propodeum joining the basal carina defining the upper limit of the propodeal declivity. Legs yellow with a brownish tinge, coxae brown. Fore Coxa two and a half times the trochanters ; femur much swollen, four times the trochanters and longer than coxa and trochanters combined ; tibia sixth-sevenths the femur ; tibial spur somewhat less than one-third the tibia and two-thirds the metatarsus ; metatarsus half the tibia and two-thirds the rest of the tarsi combined ; claw bifid (fig. 11). Hind coxa twice the trochanter; femur somewhat more than four times the trochanter ; tibia slightly longer than the femur ; tibial spur very slightly longer than one-fourth the tibia and half the metatarsus ; metatarsus half the tibia and two-thirds the rest of tarsi combined ; claw bifid (fig. 10). Wings hyaline, veins yellow. Fore wing length three times the breadth ; pro-and pterostigma light yellowish-brown ; costa and subcosta confluent ; radius distally bend, longer than pro and pterostigma combined, one-sixth the length of the wing ; basal stalk of basal nearly one-fourth the radial ; anterior branch of basal very slightly less than one and a half times the basal stalk of basal ; posterior branch of basal one and one-fourth the anterior ; basal joins the pterostigma slightly behind its middle ; the branch of basal does not enclose any cell (fig. 12). *Abdomen* smooth and shiny ; lighter in colour than the rest of the body ; only seven segments visible ; second and third segments bigger than the others ; apex of abdomen lighter in colour than the rest.

Holotype : Male dissected and mounted on slide No. $\frac{132}{8}$ labelled : Bathylid parasites of *Asphondylia morindae* Mani, from gall No. 288 on flowers of *Morinda tinctoria* Roxb., 1930. Coll : M. S. Mani, Tanjore, Madras State.

This differs from the foregoing species in having yellow legs with a brownish tinge ; antennae uniformly yellow ; hind tibial spurs more than one-fourth the tibia and interocellar slightly more than three-fourths the ocellocular.

Goniozus indicus Ashmead

1903, *Goniozus indicus*, Ashmead, *Indian Mus. Notes*, VI, p. 2.
1915, *Trissomalus fulvicornis*, Rohwer, *U. S. Nat. Mus. Proc.*, **49** : 233.
1940, *Goniozus indicus*, Muesebeck, *Proc. Ent. Soc. Washington*, **42** (6) : 121.
1942, *Goniozus indicus*, Pruthi and Mani, *Mem. Ind. Mus.*, **13** (4) : 429.

To this species I refer 3 males bred at Pusa, Bihar by C. S. Misra, on sugar-cane borers. This is known to parasitize the larvae of *Scirpophaga auriflua* Zell. ; *Cryptophlebia carpophaga* Weism. ; *Chilo* sp. on cholam and *Dialraea venosata* (Walk.).

Pristepyris agraensis, sp. nov.

Male. Length : 7mm. Colour : Black ; ant-like, with a dark reddish-brown hue in the abdomen. *Head* Viewed from above breadth two and one-fourth the length ; vertex broad, nearly smooth and shiny, convex, posteriorly margined with few long setae at the origin of which are shallow punctae ; interorbital distance five-sixths the breadth of the head ; post orbital distance nearly the same as that of the breadth of eye ; ocelli arranged in a triangle and located on the face ; ocellocular two and three-fourths the interocellar and slightly less than twice the front ocellar, front ocellus being arranged below the upper orbital border. Viewed in front length slightly more than the breadth ; interorbital distance two-thirds the breadth of the head ; face distinctly rugoso-punctate with few scattered long silvery-white setae ; gena very short, one-seventh the length of the eye ; eyes brown, bare ; post orbital distance two-thirds the length of eye ; mandibles large, strong, reddish-brown, with darker teeth ; each four toothed, the first two acute, the last two blunt ; basally attached near the lower border of the eyes. Viewed from side, temple very narrow, one-fourth the width of the eye. Antenna dark reddish-brown to brownish-black ; inserted below the middle of the eyes, on an immaginary line joining the lower orbital borders ; antennal foveae deep, broad and coriaciously sculptured ; scape slightly less than three times the pedicel ; first flagellar segment one and one-third the pedicel ; second equal to the first ; third and suceeding segments gradually become smaller, the last two segments being subequal ; the terminal segment very slightly more than one and a half times the penultimate and three-fifths the scape (fig. 16). *Thorax* Black ; length two and one-fourth the breadth near the tegulae ; prothorax long, slightly over twice the metathorax a transverse carina demarcating it into anterior and posterior areas ; anterior irregularly transversely carinate, with few scattered punctae and long setae ; posterior area comparatively smooth with few scattered punctae ; mesothorax sculpture same as the posterior part of prothorax ; parapsidal furrows deep, but posteriorly not complete ; lateral to parapsidal furrows, posteriorly are two oval foveae one on either side ; scutellum one and a half times the mesothorax ; slightly convex, with few, large, distinct, scattered punctae and few long setae ; there are broad foveae two on either side of the scutellum ; metathorax one-third the scutellum, with many deep foveae ; propodeum slightly longer than metathorax, scutellum and metathorax combined, anteriorly depressed on either side, below which laterally are placed the spiracles which are large elongate-oval ; the anterior depressions are longitudinally carinate ; propodeum reticulately rugoso-sculptured, rounded posteriorly without basal or lateral carinae. Legs dark reddish-brown to black ; except comparatively lighter trochanter and tarsi. Fore coxa nearly one and one-fifth the trochanter ; tibia four-fifths the femur ; tibial spur larger one slightly more than one-third the tibia ; smaller one-third the larger ; metathorax three-fifths the tibia and somewhat less than three-fourths the rest of tarsi combined ; claw trifid (fig. 17). Hind coxa slightly over twice the trochanters ; femur somewhat less than twice the coxa and nearly four times the trochanters ; tibia a little longer than the femur ; metatarsus a little over half the tibia ; rest of tarsi one and two-thirds the metatarsus ; claw trifid, the third tooth blunt (figs. 14 & 15). Wings : Length of fore wing more than two and a half times the breadth ; very distinct cell between subcosta and costa ; basal part of pterostigma hyaline ; prostigma absent ; metacarp one-fourth the radius radius one-fourth the length of the wing ; basal somewhat less than half the radius ; transversal four-fifths the basal (fig. 12 and 13). *Abdomen* nearly the same length as the thorax, petiolate ; petiole one-eighth the rest of abdomen ; elongate, basally slightly bend, dorsally slightly convex ; apex narrow, but truncate ; basally and dorsally smooth, shiny and bare ; laterally and at apex covered over by silvery-white pubescence, with an yellowish tinge ; first tergite much larger than the rest ; rest of them become shorter.

Holotype : Male on pin No. $\frac{5}{B}$ partly dissected and mounted on slide No. $\frac{5}{B}$ labelled : on wing, Botanical Garden, St : John's College, Agra ; Coll : Chandy Kurian, 10-viii-1949, Agra.

This is the first record of the genus from India.

Pristocera govindrami, sp. nov.

Male. Length : 8.5 mm. Dark reddish-brown to black, *Head* black, nearly prognathous ; viewed from above two and one-fourth

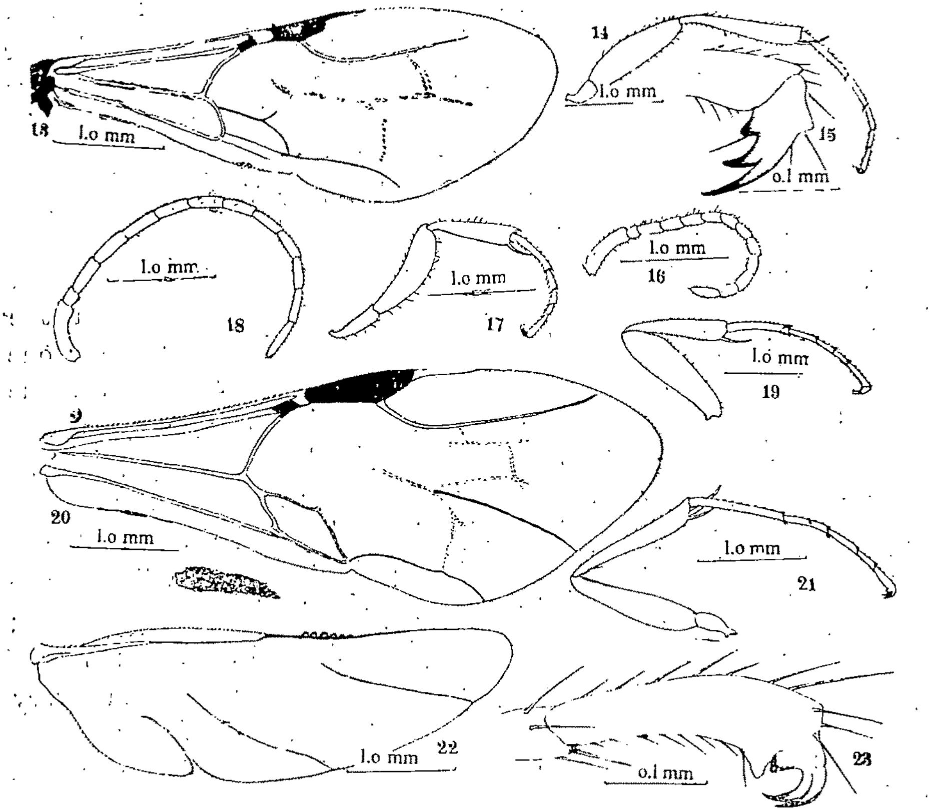

FIG. 13–17. *Pristepyris agraensis,* sp. nov. Male. 13. fore wing, 14. hind leg, 15. claw of hind leg enlarged. 16. antenna, 17. fore leg.

FIG. 18–23. *Pristocera govindrami,* sp. nov. Male. 18. antenna, 19. fore leg, 20. fore wing, 21. hind leg, 22. hind wing, 23. claw of hind leg enlarged.

as broad as long ; interorbital two-thirds the width of the head ; vertex convex, margined, with large scattered punctae and long silvery-white pubescence ; ocelli reddish-brown arranged in a triangle ; interocellar slightly more than the front ocellar and slightly more than half the ocellocular which latter is two and a half times the front ocellar ; viewed in front nearly quadrate, width one and one-sixth the length. Eyes slightly diverge posteriorly ; interorbital distance somewhat less than two-thirds the width of the head ; frons and face distinctly rugoso-punctate ; punctae large and deep ; length of eye nearly five times that of the short gena ; mandibles dark reddish-brown, with darker teeth ; four toothed ; first acute and the rest blunt. Antenna inserted below the level of the eye one on either side of the clypeus ; brownish-black except for the dark reddish-brown scape and pedicel ; scape three and a half times the pedicel ; pedicel a little longer than broad ; first flagellar segment two and a half times the pedicel ; second and the remaining segments gradually become shorter ; penultimate three-fourths the first flagellar ; terminal nearly one and one-third the penultimate, equal to the first flagellar and slightly more than two-thirds the scape (fig. 18). *Thorax* black ; prothorax anteriorly slightly slanting ; distinctly rugoso-punctate ; punctae large, coalesed and scattered far apart in the middle and more thickly arranged at the sides ; mesothorax half the prothorax, sculpture irregular, with few shallow, indistinct punctae ; parapsidal furrows broad, deep and complete ; scutellum nearly flattened, nearly same length as the mesothorax and punctation also nearly the same ; two broad foveae on either side ; propodeum nearly the same length as mesothorax, scutellum and metathorax combined ; convex, posteriorly steeply sloping ; roughly transversely carinate, with a few longitudinal connecting carinae which give a nearly reticulately-carinate appearance ; propodeal spiracles small and lanceolate. Legs somewhat dark reddish-brown with comparatively lighter trochanters ; fore coxa slightly longer than the trochanter ; femur nearly twice that of trochanter ; tibia five-sixths the femur ; tibial spur slightly longer than one-third the tibia ; metatarsus slightly more than three-fifths the tibia and nearly two-thirds the rest of tarsi combined ; claw trifid (fig. 19). Hind coxa one and three-fourths the trochanter ; femur nearly four times the trochanter ; tibia very slightly longer or nearly equal to femur ; tibial spur, the larger one slightly less than one third the tibia ; smaller somewhat more than half the larger ; metatarsus five-sevenths the tibia and somewhat more than five-sevenths the rest of the tarsi combined ; claw trifid (fig. 21 and 23). Fore wing length nearly two and two-thirds the breadth ; radius one-fourth the length of the wing ; basal half the radius ; transversal somewhat more than three-fourths the basal ; prostigma absent ; post marginal vein a little less than half as long as stigma (fig. 20 and 22). *Abdomen* Dark reddish-brown to black nearly the same length as the thorax ; elongate ; slightly convex, posteriorly narrowing, but apex somewhat truncate ; smooth and shiny ; basally and dorsally nearly bare, but laterally and at apex covered by long silvery-white pubescence, with a yellowish tinge.

Holotype : Male on pin No. $\frac{7}{8}$ partly dissected and mounted on slide No. $\frac{7}{8}$, labelled : a bethylid parasite on window, coll : G. R. Dutt ; 23-vi-1920, Suduganga.

This species is closely related to *Pristocera areolata* Muesebeck, but differs in having the pedicel a little longer than broad ; first flagellar segment only two-thirds the scape ; median longitudinal carina incomplete and post marginal vein being a little less than half as long as stigma.

CONTRIBUTIONS TO OUR KNOWLEDGE OF FIG-INSECTS (CHALCIDOIDEA : PARASITIC HYMENOPTERA) FROM INDIA*

II—On a new species of *Lipothymus* Grandi from *Ficus hispida* Linn.

By K.J. Joseph, M.Sc., *Government of India, Ministry of Education, Senior Research Scholar, School of Entomology, St. John's College, Agra.*

The type specimens of the new forms described here are kept in the collections of the School of Entomology for the time being.

I am very thankful to Prof. M.S. Mani for facilities for work, guidance and confirmation of my identifications.

Lipothymus bakeri, sp. nov.

Female.—Length 1.9 mm. Iridescent green with a fundamental yellowish-brown colour for the whole of the ventral side; legs and antennal scape brownish-yellow, rest of antenna sooty-brown. Wings hyaline, nervures amber; ovipositor flavo-testaceous, valves infuscate.

Head (Fig. 1) markedly wider between the external margins of the eyes than long; epistomal margin excurvo-emarginate; lateral margin forwards of eyes (gena) a little less than half the longitudinal diameter of the eye. A V-shaped groove present on the head, but this is not so well-pronounced as in *L. glomeratus* Joseph[1]. Eyes large, chocolate-brown; ocelli three in a large obtuse triangle, pale brown; posterior margin of head behind the eyes a little concave, head with a few setae and definite leathery sculpture. Antenna (Fig. 2) twelve-segmented, the third and the fourth segments forming the ring-joints, the tenth, eleventh and twelfth segments fused to form a club; scape thickest in the middle, about five times as long as its maximum thickness and about equal

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[1] In the press (Ind. J. Ent.).

to the five following segments combined, with setae as in figure; second segment one-third as long as scape and one and two-thirds as long as its own thickness, narrow at base, increasing in thickness towards apex, with a few short and thick setae; second ring-joint larger than the first and with a whorl of setae; fifth segment longer than thick; sixth a little shorter than fifth, longer than its own thickness; seventh equal to fifth; eighth longer than seventh, one and a half times as long as its own thickness; ninth a little shorter than eighth, but a little larger than seventh, a trifle less than one and a half times as long as its own thickness; segments five to nine of the same thickness; club a little less than three and a half times as long as its maximum thickness, this latter being equal to the thickness of segments five to nine; segments five to twelve bear whorls of long sensoria and long setae as in figure. Mandible (Fig. 3) a little over one and two-thirds as long as broad, bidentate with stout and acute apical tooth and stouter and blunt subapical tooth, setae as in figure. Maxillary palpus four-

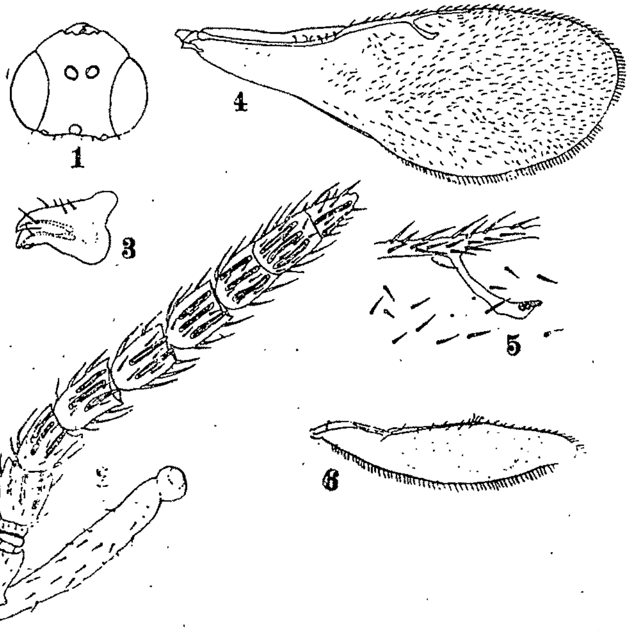


FIG. 1. *Lipothymus bakeri*, sp. nov. Female. Head.
FIG. 2. *Lipothymus bakeri*, sp. nov. Female. Antenna.
FIG. 3. *Lipothymus bakeri*, sp. nov. Female. Mandible.
FIG. 4. *Lipothymus bakeri*, sp. nov. Female. Fore wing.
FIG. 5. *Lipothymus bakeri*, sp. nov. Female. Details of stigmal vein.
FIG. 6. *Lipothymus bakeri*, sp. nov. Female. Hind wing.

segmented with the lengths of the segments beginning from the base in the proportion 8 : 9 : 6 : 6·5. Labial palpus two-segmented, their proportion, beginning at the base being 6·5 : 6.

Thorax with fine leathery sculpture and small setae. Pronotum markedly wider than long, with a slight incurvation anteriorly. Mesonotum with scapulae broader than long, with rounded external margins; scutum broader than long; parapsidal furrows complete and deep, arched inwards; scutellum subovoidal, longer than wide, its lateral margins parallel in the posterior half, rounded off in the anterior half, parascutellum small, longer than wide. Metanotum a narrow transverse area. Wings. Fore wings (Fig. 4) a little less than two and a half times as long as wide, rounded off at the distal extremity, costal cell about eleven times as long as its own width, bearing about

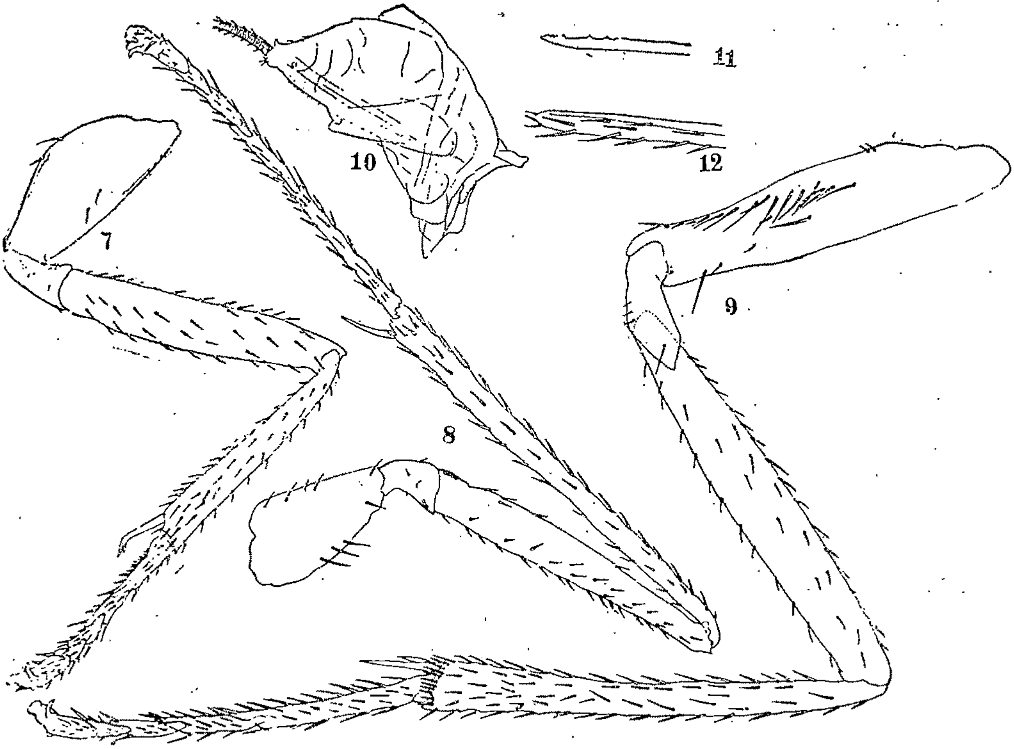


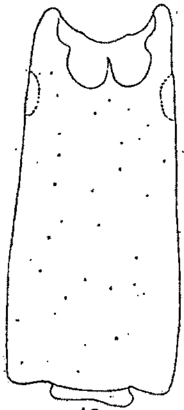
FIG. 7. *Lipothymus bakeri*, sp. nov. Female. Fore leg.
FIG. 8. *Lipothymus bakeri*, sp. nov. Female. Mid leg.
FIG. 9. *Lipothymus bakeri*, sp. nov. Female. Hind leg.
FIG. 10. *Lipothymus bakeri*, sp. nov. Female. Abdomen, lateral view.
FIG. 11. *Lipothymus bakeri*, sp. nov. Female. Tip of ovipositor.
FIG. 12. *Lipothymus bakeri*, sp. nov. Female. Valve, distal part.

six setae; submarginal vein about twice as long as the marginal and about two and a half times as long as the postmarginal; marginal vein one and one-fourth postmarginal; stigmal half postmarginal and oblique with respect to the costal margin and tilted towards the distal extremity, subclaviform, with a beak-like prolongation distally bearing a set of four sensillae as in fig. 5; alar cuticle pubescent, pubescence brownish-black, short and thick, a single linear pattern in the disposition of the pubescence clearly seen in the posterior region as in figure; marginal fringe moderately large-sized. Hind wing (Fig. 6) about four and a half times as long as wide; alar cuticle pubescent, pubescence brownish-black and extremely short, with three frenal hooks; marginal fringe long. Fore leg (Fig. 7) with coxa subtriangular, two and three-fourths as long as broad, with setae as in figure; trochanter longer than its maximum width, with a few setae; femur long, six times as long as wide, with short setae; tibia shorter than femur, a little over seven times its own maximum width, with many setae and distally with a long bifid tibial spur; tarsi five-segmented, armed with setae and spines, metatarsus a little less than the two following segments combined, bearing on its ventral margin about ten setae set in a comb-like series, combined length of fourth and fifth segments markedly less than the metatarsus, fifth segment equal to half the combined length of the second and third segments; pretarsus with a pair of curved claws and empodium. Mid leg (Fig. 8) with coxa subtriangular, twice as long as broad, with setae as in figure; trochanter one and two-thirds as long as broad; femur long, a little less than eight times as long as broad, with setae as in figure; tibia very long, longer than the femur, about sixteen times as long as its width, with many setae and a long apical spur; tarsi long, five-segmented, armed with setae and spines, metatarsus one and one-third as long as the two following segments combined, and two and a half times as long as the fourth and the fifth segments united, a little over four times the fifth segment; pretarsus with a pair of curved claws and empodium. Hind leg (Fig. 9) with coxa very long but shorter than the femur and as in figure, a little less than five times as long as its width, bearing about twenty-three long brownish-red setae on its inner side; trochanter one and two-fifths as long as wide, with a few small setae; femur about six and a half times as long as its width, with setae; tibia long, longer than the femur, about eight times as long as wide, with many long setae and spines all over, a fringe of about ten spines along its distal extremity and a pair of apical spurs of unequal lengths as in figure; tarsi about as long as the mid tarsi, five-segmented, armed with spines and setae, metatarsus a little over half the length of the tarsi, longer than the combined length of the two following segments, fourth and fifth segments combined a little less than half the metatarsus, fifth about one-third the combined length of the second and third segments; pretarsus with a pair of curved claws and the empodium. Propodeum about two and a half times as broad as long, anterior margin faintly and posterior margin deeply emarginate, laterally gently narrowing posteriorly, with a few setae postero-laterally; a pair of tracheal spiracles provided with rather small subovoid peritremes situated laterally.

Abdomen as in figure 10, two and a half times as long as wide, sternites of segments 2, 3 and 4 of the abdomen produced antero-ventrally to form a keel-like structure; eighth urotergite with a pair of tracheal spiracles furnished with small subrotund peritremes; ninth segment small, with a pair of cercoides bearing setae; tenth reduced and membranous; ovipositor (Fig. 11) three and one fourth as long as the abdomen; valves (Fig. 12) setiferous and gently swollen apically.

Male.—Length 2·6 mm. Head, thorax and legs fulvous, mandibles and scape and second segment of the antenna ferrugino-testaceous, abdomen and the third segment of the antenna pallide-flavens, eyes brownish-black.

Head (Fig. 13) about three times as long as broad, lateral margins substraight, faintly converging towards the anterior end, posterior margin as in figure; epistomal margin incurved and emarginate; eyes small. Antenna (Fig. 14) inserted in an anterior depression of the head, three-segmented; scape a little less than thrice as long as thick; second segment narrow at base, broad distally, twice as long as its maximum thickness; third segment subconical; one and two-thirds as long as thick, with a few sensoria apically. Mandible (Fig. 15) a little less than twice as long as broad, bidentate with long, acute and falcate apical tooth and stout and blunt subapical tooth, with setae as in figure.



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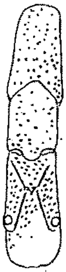

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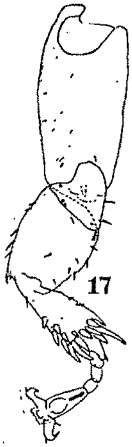

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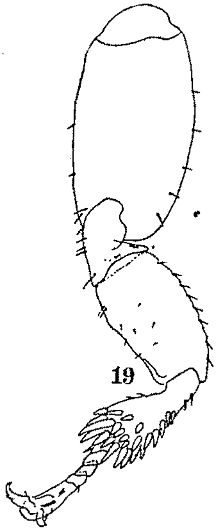

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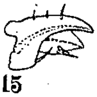

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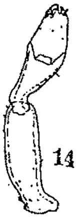

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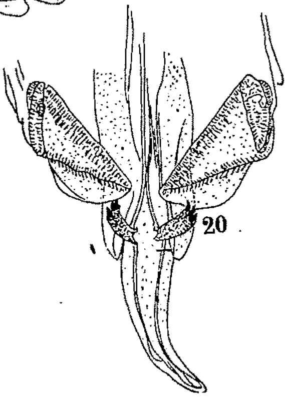

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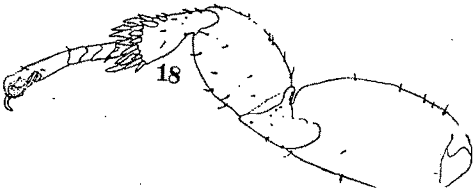

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FIG. 13. *Lipothymus bakeri,* sp. nov. Male. Head.
FIG. 14. *Lipothymus bakeri,* sp. nov. Male. Antenna.
FIG. 15. *Lipothymus bakeri,* sp. nov. Male. Mandible.
FIG. 16. *Lipothymus bakeri,* sp. nov. Male. Thorax & propodeum.
FIG. 17. *Lipothymus bakeri,* sp. nov. Male. Fore leg.
FIG. 18. *Lipothymus bakeri,* sp. nov. Male. Mid leg.
FIG. 19. *Lipothymus bakeri,* sp. nov. Male. Hind leg.
FIG. 20. *Lipothymus bakeri,* sp. nov. Male. Abdomen, details of spiracles, cerci and, penes.

Thorax (Fig. 16) Pronotum elongated, about twice as long as its maximum width, lateral margins straight, but converging forwards, anterior margin somewhat rounded off, posterior margin deeply concave, with a few very minute setae; mesonotum as in figure; metanoto-propodeum a little over twice as long as wide, with minute setae and punctulae, propodeal tracheal spiracles with large subovoid peritremes situated laterally. Fore leg (Fig. 17) with coxa long, over twice as long as broad; trochanter about as long as broad; femur widest in the middle, converging towards apex, about one and a half times as long as wide, with a few sensillae and setae as in figure; tibia a little over twice as long as broad, with a few setae and with many odontoid spines laterally on the external margin and distally; tarsi five-segmented, almost unarmed and as in figure, metatarsus a little less than the combined length of the second and third segments, fourth and fifth segments combined equal to twice the united length of the second and third segments, fifth segment twice as long as the metatarsus and a little over twice as long as its own breadth; pretarsus with a pair of large curved claws and the empodium. Mid leg (Fig. 18) with coxa as long as the fore coxa but stouter, twice as long as broad, outer margin curved, inner margin substraight with a few setae; trochanter over one and a half times as long as broad, with setae as in figure; femur widest in the middle, converging towards apex, about one and a half times as long as broad, with a few sensillae and setae; tibia twice as long as broad, with setae and with odontoid spines distally and laterally on the external margin; tarsi five-segmented, equal to the fore tarsi, almost unarmed and as in figure; metatarsus equal to the fore metatarsus, second and third segments combined a little less than twice the metatarsus, fourth and fifth segments combined equal to one and a half times the combined length of the second and third segments, fifth segment equal to half the combined length of all the tarsal segments and a little less than thrice its own breadth; pretarsus with a pair of large curved claws and the empodium. Hind leg (Fig. 19) with coxa large, larger than the fore and mid coxae, subelliptic, twice as long as broad, with a few long setae; trochanter a little less than one and a half times as long as broad, with a few setae; femur large and as in figure, about one and a half times as long as broad, with setae mostly along its outer margin; tibia twice as long as broad, with a few setae and large odontoid spines distally and laterally on the external margin; tarsi five-segmented, a little shorter than the fore and the mid tarsi, almost unarmed and as in figure, metatarsus equal to those of the fore and the mid tarsi, second and third segments combined equal to the fifth segment, and about two-thirds the combined length of the fourth and the fifth segments; pretarsus with a pair of large claws and empodium.

Abdomen a little over thrice as long as wide ; eighth segment with a pair of spiracles provided with very large pinna-like peritremes (Fig. 20) ; ninth and tenth segments tubular, tenth with a pair of anal cerci each of which is provided with three claw-like teeth ; penes exserted and as in figure.

Holotype 1 female dissected on slides Nos. 13/4-1, 13/4-2, 13/4-3, 13/4-4, *allotype* 1 male dissected on slides Nos. 13/2-1, 13/2-2, 13/2-3, 13/2-4, 13/2-5. Paratypes females & males in spirit. Collected by K. J. Joseph from figs of *Ficus hispida* LINN., from Baker Memorial School Compound, Kottayam, South India. 26-vi-1951.

L. Sumatranus Grandi[2] described from female only from Sumatra differs from *L. bakeri,* sp. nov. in the following chief characters :—

(1) Head a little wider between the external margins of the eyes than long. Lateral margin forwards of eyes a little shorter than the maximum diameter of the eyes.

(2) Antennal scape about 4 times as long as its greatest width and a little longer than the 5 following segments combined.

(3) Along the dorsal margin of the mandible are found about twenty specialized setae.

(4) Maxillary palpus with first and second segments almost of equal length, third shortest of all, fourth longest and about equal to the combined length of the first and second segments. Labial palpus with the segments of equal length.

(5) Fore wings about twice as long as their maximum width ; costal cell a little more than seven times its width and furnished with three setae. Submarginal vein about one and one-third as long as the marginal, marginal vein one and a half postmarginal, postmarginal less than twice as long as the stigmal. Hind wings almost four times as long as their maximum width.

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[2] Grandi, G. 1921. *Ann. Mus. Civ. Stor. Nat. Genova,* ser. (3a) **9** : 307-308 ; Grandi, G.1922. *Boll. Lab. Zool. Portici,* **15** : 218-222, Fig. VI-VII; Grandi, G.1941. *Boll. Ent. R. Univ. Bologna,* **13** : 24.

(6) Few odontoid spines on the fore tibia and about forty on the hind tibia. Hind coxa longer than the femur and about one and a half times its breadth. Hind metatarsus with a series of five ventral submarginal odontoid spines.

L. glomeratus Joseph described from females & males, from Agra differs from *L. bakeri*, sp. nov. in the following chief characters :—

(a) *Female.*

(1) Lateral margin forwards of eyes about half the longitudinal diameter of the eye ; a prominent V-shaped groove on the head.

(2) Antennal scape slightly longer than the five following segments combined.

(3) Maxillary palpus with the segments in the ratio 7:9:6:10. Labial palpus with two equal segments.

(4) Fore wings about two and a half times as long as maximum width ; costal cell about nine times its width and furnished with nine setae ; submarginal vein about two and a half times as long as the marginal and thrice as long as the postmarginal ; marginal about one and one-fifth postmarginal ; stigmal less than half the postmarginal ; pila in the posterior half in three linear patterns ; hind wings about four times as long as its maximum width.

(5) Fore metatarsus ventrally with a row of thirteen spines submarginally arranged in a comb-like series ; fore and hind tibiae devoid of odontoid spines ; hind coxa over thrice as long as thick and clearly shorter than the femur.

(b) *Male.*

(6) Head over twice as long as wide.

(7) Antennal scape thrice as long as thick.

(8) Pronotum less than two and a half times as long as wide ; mesonotum bears in its posterior region a clearly demarcated area comparable to the scutellum of the female.

(9) Fore coxa about twice as long as broad ; tibia over one and a half times as long as its maximum width ; mid coxa shorter than the fore coxa ; hind coxa less than twice as long as broad.

In view of the above differences it has to be concluded that *L. bakeri*, sp. nov. is more related to *L. glomeratus* Joseph than to *L. sumatranus* Grandi.

ON SOME ARTHROPOD MICROFOSSILS FROM INDIA

By V.P. GEORGE, M.Sc., *Lecturer in Zoology, St. John's College, Agra.*

Arthropod microfossils from India were first discoverd by Sahni[1] and described by Mani.[2] Among the many interesting fragments brought to light by these two workers, *Chironomus primitivus* Mani (*loc. cit.*) has created a great deal of interest in view of the light it throws on the age of the Saline Series, Salt Range, Punjab. Another striking remain refers to *Gamasus fossilis* Mani (*loc. cit.*), which was discovered by Sahni from the Worli Hills, Bombay. In the present paper, I deal with forty-six more or less complete, often extremely well preserved, sclerotized fragments of the exoskeleton of various arthropods, partly from the Saline Series and partly from the Cretaceous Limestone-clay of the Inter-Trappean of Nagpur.

I take this opportunity of recording my grateful thanks to Prof. M. S. Mani, Professor of Zoology & Entomology, School of Entomology, St. John's College, Agra, for placing the interesting material at my disposal, for guidance and facilities for work.

MICROFOSSILS FROM THE SALINE SERIES, PUNJAB[3]

Class ARACHNOIDEA

Order *ACARINA*

Family PARASITIDAE

Fragment No. 1.

A nearly complete specimen (Fig. 16) of the protonymph of a free-living mite, possibly of the Gamasids. Body nearly transpar-

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*Contribution No. 28 from the School of Entomology, published with the permission of the Professor of Zoology & Entomology.

[1] Sahni, B. & B. S. Trivedi. 1943. *Abstr. Proc. Joint Meet. nat. Acad. Sci. India, Hyderabad*, pp. 25-26 ; Sahni, B. 1944. *Proc. nat. Acad. Sci. India*, 14 (6) : i-xxxii.

[2] Mani, M. S. 1945. *Indian J. Ent.*, 6 : 61-64a, *Proc. nat. Acad. Sci. India*, Udaipur Symposium, 16 (2-4) :43-56.

[3] Now in Pakistan.

ent, but infiltered with the granules of the matrix, so that it is difficult to observe the position and the structure of the genital operculum and genital aperture. The chelate mandibles and the finely hooked gnathal appendages and the slender curved claws are well preserved; two legs of one side are missing; the terminal segments of the legs are slender on the hind and the third leg, but somewhat stouter in others; claw a little less than half the distal segment of the leg. Length 240 micra.

Slide No. 9. From dolomite from the base of the Saline Series at the junction of Jankush Nulla and Warcha Gorge, Warcha. Coll. B.S. Lamba. 1945.

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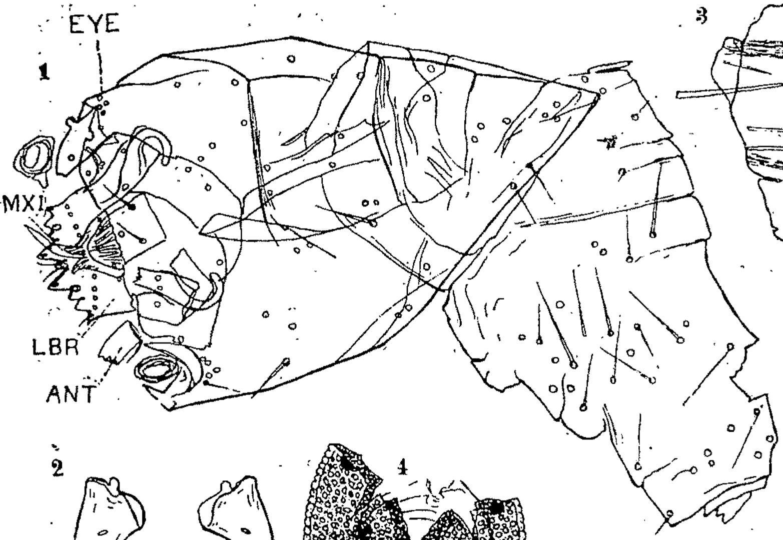

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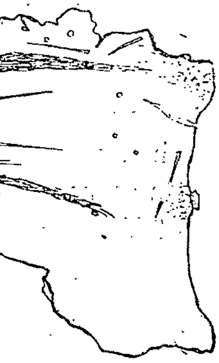

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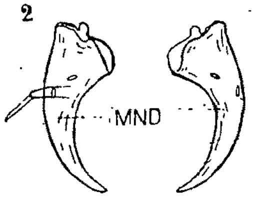

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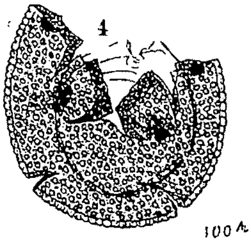

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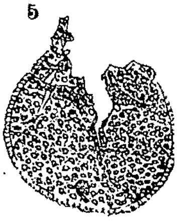

FIG. 1. Head capsule and part of thorax and abdomen of a Dytiscid larva. Ant. Antenna; EYE. Compound eye; LBR. Labrum; MXI. Maxilla.

FIG. 2. Mandibles of a Dytiscid larva. MND. Mandible.

FIG. 3. A piece of chitinous arthropodan exoskeleton.

FIG. 4. Empty egg shell of an unidentified Arthropod.

FIG. 5. Empty egg shell of an unidentified Arthropod.

An incomplete specimen of protonymph of another Gamasid mite was described by Mani* from Warcha. This additional find is of great interest.

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*Mani, M.S. 1946. *Proc. nat. Acad. Sci. India*, 16 (2-4): 52. Fig. 1,3.

Order CORRODENTIA

Fragment No. 2.

Head, thorax and anterior part of the abdomen of an extinct apterous psocid (Fig 59) in excellent state of preservation. The head nearly complete, 265 micra long. Mouth parts with the two mandibles (Fig. 58, MND) distinct ; the mandibles moderately sclerotized, with the condyles conspicuous ; triangular ; the length from the tip of the incisor tooth to the condyle one and one-fourth the maximum width, with six distinct teeth ; apical tooth acute ; the molar teeth small and acute ; the first molar slightly larger than the second, third nearly equal to the second ; the molars broad and denticulate, the first two denticles acute ; the last broad and blunt. A part of labrum (Fig. 59, LBR) is also preserved. The maxilla represented by the characteristic bidentate lacinia or maxillary pick (Fig. 59, MXIPX) ; length of maxillary pick nine times its greatest thickness ; one tooth twice as long as the other. Labium (Fig. 59, MX2) completely preserved. Both the compound eyes (Fig. 59, EYE) are present but vestigial ; each represented by a loose group of six or seven ommatidia ; eyes 140 micra in diameter, ommatidium 30 micra. Ocelli absent. The thorax is damaged but the peculiarities of the various sclerites characteristic of the order are easily made out ; the prothorax complete with the foreleg *in situ*. The fragments of the basal part of the third leg of one side (Fig. 59, LG 3) are represented by both coxae and parts of trochanter. The pleuron is well preserved. The pleural suture is distinct and the differentiation of mesepisternum and mesepimeron is fairly clear. Abdomen represented by six distinct basal segments. Length of the entire specimen 0. 8 mm.

Fragment No. 3.

On the same slide is mounted the hind leg of a psocid (Fig. 58) with a fragment of femur, complete tibia and tarsus. The enormously swollen saltatorial hind femur, together with the triarticulate tarsus, readily identify the fragment. The femur is nearly as long as wide ; the basal part and one of its lateral surfaces are missing. The tibia is somewhat shorter than the femur, with a length seven times its diameter. The tarsus nearly three-fourths the tibia ; metatarsus with a length four times its thickness ; second tarsal segment pyriform two-thirds the metatarsus ; third tarsal segment slender, a little shorter than the metatarsus and nearly twice as long as thick. Claw stout, with the other related structures damaged. The apex of the tibia bears a pair of stout, slightly curved, tooth-like spines. The lower margin of the apical half of the tibia and apices of the tarsal segments with fine elongate spines. Femur 100 micra thick ; tibia 100 micra long.

Slide No. 22. From Marl, Low level tunnel, Warcha Mine, Chamber No. 21 between the top and the next seam. Coll. B. S. Lamba 1945.

Inspite of the fragmentary nature of the material and the fact that it is partly crushed and flattened out, the characteristic mandible, the maxillary pick and the thoracic sclerites are unmistakable. It is not possible, however, to place the specimen more difinitely in suborder or family, and much less genus.

The order is comparatively a recent one and belongs to a specialized branch of the descendants of an ancient blattoid stock. The Corrodentia, together with the Mallophaga and Anoplura, are often placed together in Blattaeformia. Living species are abundant nearly throughout the world but more particularly in the humid tropical regions. They occur under fallen leaves, on barks and old undisturbed records.

Order COLEOPTERA
Suborder *POLYPHAGA*
Family DERMESTIDAE

Fragment No. 4.

Disintegrated fragment of the exoskeleton of the larva of a Dermestid beetle (Fig. 38). The head completely detached from the body ; head capsule (Fig. 40,43) represented by a narrow piece of the epistomal suture, with the clypeus and the quadrate labrum *in situ.* Mandibles (Fig. 39,41 MND) with condyles and heavily sclerotized apices ; maxillae (Fig. 39 MXI) well preserved but

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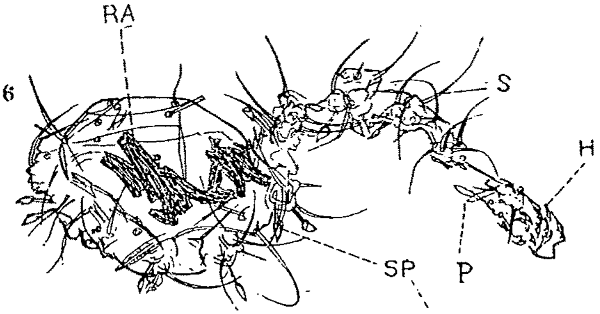

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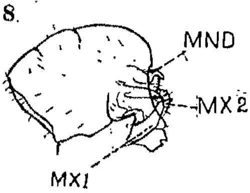

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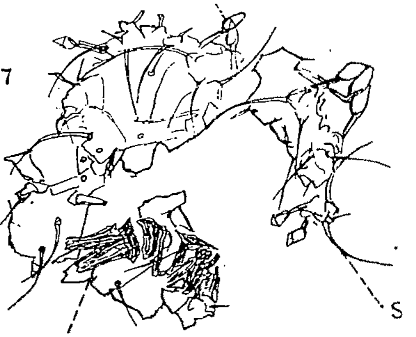

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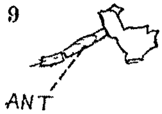

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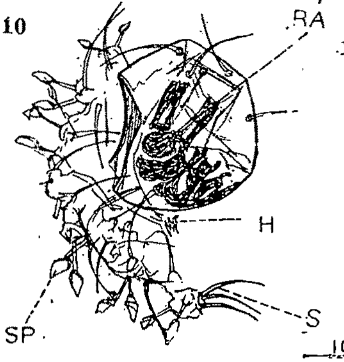

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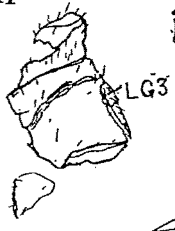

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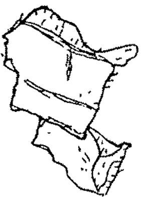

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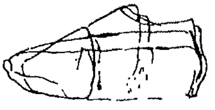

FIG. 6. Pupa of a Nematocerous Diprera. H. Anchoring hooks ; P. Paddles ; RA. Respiratory armature ; S. spine ; SP. Spoon-shaped structures.

FIG. 7. Pupa of a Nematocerous Diptera (Partly damaged) RA. Respiratory armature ; S. Spine ; SP. Spoon-shaped structures.

FIG. 8. Cephalic region of a Collembolan. MND Mandible : MX1. Maxilla ; MX2. Labium.

FIG. 9. Part of antenna of a Collembolan. ANT. Antenna.

FIG. 10. Pupa of a Nematocerous Diptera. H. Anchoring hook ; RA. Respiratory armature ; S. Spine ; SP. Spoon-shaped structure.

FIG. 11. Part of thorax of a Collembolan. LG3. Hindleg.

FIG. 12. Part of abdomen of a Collembolan.

FIG. 13. Chitinous exoskeleton of an Arthropod.

detached from the main piece ; galea typically biarticulate ; lacinia with well preserved comb of brush-like setae ; cardo and part of stipes are also preserved. Body with numerous long, spiny, barbed setae and stout erect spines : setae in bunches, especially posteriorly. The exact number of abdominal segments cannot be accurately counted. Three legs (Fig 42) of one side are preserved, of which the foreleg (Fig. 42 $^{\mathrm{LG1}}$) is complete with the claw, which latter is five times as long as thick ; in the midleg (Fig. 42, LG2) the claw is entirely lost and in the hindleg (Fig. 42, $^{\mathrm{LG3}}$) the tip of the claw is broken. Mandibles length 200 micra, width 200 micra ; maxilla length 160 micra ; labrum half as long as wide, width 160 micra.

Slide No. 18. From dolomite from the base of the Saline Series at the junction of Jankush Nulla and Warcha Gorge, Warcha. Coll. B.S. Lamba 1945.

The nearly complete mouthparts, the leg and the characteristic setae and spines are unmistakable diagnostic characters of the dermestid larvae.

Mani* has described a single hair of a dermestid larva.

Fragment No. 5.

A single barbed seta of a dermestid larva (Fig. 45) ; long and slender, light brown, solid, stout basally, somewhat darker at the base.

Slide No. 23. From Marl. Low level tunnel, Warcha Mine, Chamber No. 21 between the top and the next seam. Coll. B.S. Lamba 1945.

Order LEPIDOPTERA

Family SPHINGIDAE

Fragment No. 6.

A single complete scale from the wing of a moth probably Sphingidae (Fig. 55), long and narrow ; apically with three small subtriangular lobes ; with fine longitudinal striations ; slightly damaged beyond the middle ; about ten times as long as the maximum width ; pale hyaline-brown. Total length 640 micra.

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*Mani, M.S. 1946. *Proc. nat. Acad. Sci. India*, 16 (2-4) : 52.

Although the extreme base, particularly the root of the scale, is broken and no trace of the original colour is left, the unmistakable sphingid facies can be easily made out. The family Shingidae is a Recent group.

Slide No. 16. From dolomite from the base of the Saline Series at the junction of Jankush Nulla and Warcha Gorge, Warcha. Coll. B.S. Lamba 1945.

Order COLLEMBOLA

Fragment No. 7.

An incomplete specimen of a Collembola (Fig. 8,9,11,12) with the integumental pigmentation still preserved. While mounting the fragment broke into three or four pieces on the slide. The largest piece (Fig. 8) includes the head with the endognathous mouthparts and part of the thorax. The single mandible (Fig. 8 $^{\mathrm{MND}}$) curved and pointed ; maxilla (Fig. 8 $^{\mathrm{MXI}}$) of both sides well preserved ; curved inwards with serrated distal ends ; labium (Fig. 8. $^{\mathrm{MX2}}$) flat and broad with stout bristles ; part of the terminal segment of one antenna (Fig. 9) is also preserved. A second piece (Fig. 11) comprises the damaged part of the thorax and abdomen with the unmistakable collembollan hindleg (Fig. 11 $_{\mathrm{LG3}}$). The third piece (Fig. 12) is a part of abdomen with a few integumental bristles and irregular patches of the characteristic pigments. The first piece with head and part of thorax 200 micra long ; second, with one leg 85 micra long and third piece 210 micra long.

Slide No. 45. From Marl. Low level tunnel, Warcha Mine, Chamber No. 21 between the top and the next seam. Coll. B. S. Lamba, 1945.

A relatively large piece of Collembola was described by Mani.*

The Collembola are apterous and primitive in many respects and are devoid of scaly covering on the body, with usually fused

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*Mani, M.S. 1946. *Proc. nat. Acad. Sci. India*, 16 (2-4) : 44.

thoracic segments, short and similar legs, with no spiracles or tracheal system. They are, however, not an ancient order and are

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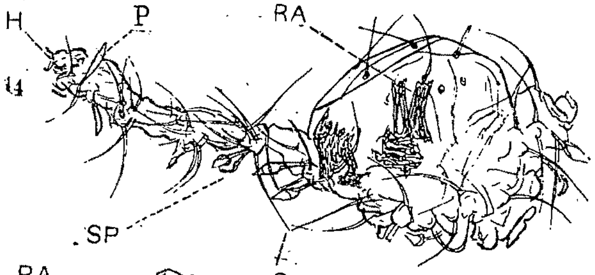

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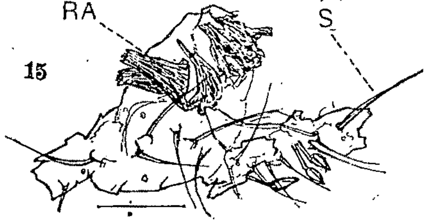

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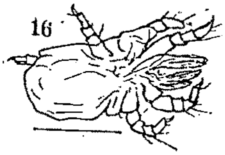

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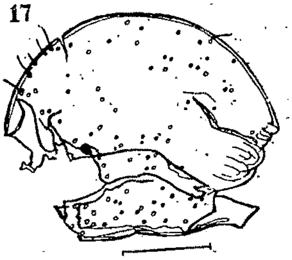

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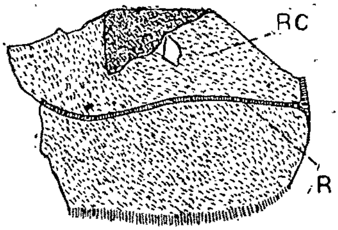

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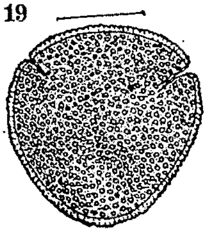

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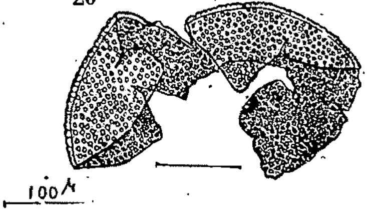

FIG. 14. Pupa of a Nematocerous Diptera. H. Anchoring hooks; P. Paddles; RA. Respiratory armature ; S. Spine ; SP. Spoon-shaped structure.
FIG. 15. Part of the exoskeleton of the pupa of a Nematocerous Diptera; RA. Respiratory armature; S. Spoon-shaped structure.
FIG. 16. Protonymph of a free-living Gamasid mite.
FIG. 17. Part of cephalic capsule of an Arthropod.
FIG. 18. Posterior and subapical part of the fore-wing of an Ichneumonid; R. Radial vein; RC. Radial cell.
FIG. 19. Empty egg shell of an unidentified Arthropod.
FIG. 20. Empty egg shell of an unidentified Arthropod.

believed to have evolved from the Protephemeroid stem of the Pterygogenia during the Cretaceous. The oldest fossil Collembola comes from the Tertiary. Sixty seven species are known from the

Baltic Amber (Oligocene) and four from the Copal (Pleistocene). Recent forms are widely distributed.

INCERTAE SEDIS

Fragment No. 8.

Two emarginate chitinous fragments of the exoskeleton of an arthropod (Fig. 61), subconical, nearly twice as long as wide, dark brown, without any sculpture or other cuticular sensory structure. Length 480 micra.

Slide No. 10. From dolomite from the base of the Saline Series at the junction of Jankush Nulla and Warcha Gorge, Warcha. Coll. B. S. Lamba 1945.

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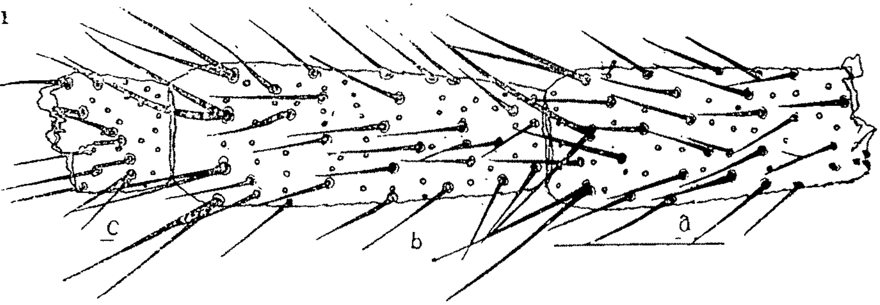

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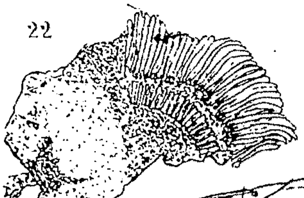

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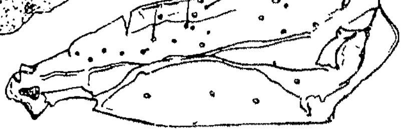

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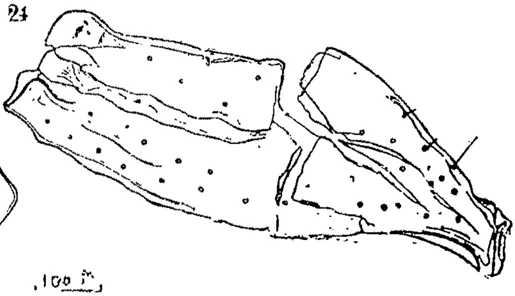

FIG. 21. Part of an antenna of a Carabid beetle.
FIG. 22. Part of the compound eye of an Arthropod.
FIG. 23. Pedicel of the antenna of a Dermaptera.
FIG. 24. Scape of the antenna of a Dermaptera.

Fragment No. 9.

Part of the cephalic capsule of an arthropod (Fig. 17) with the oral end nearly well preserved and part of the postoccipital integumental inflexion and the mandibular condyle distinct. A very faint epistomal suture can also be made out. The entire fragment is covered by fine setae. Colour amber-yellow. Length 245 micra. Width 240 micra.

Slide No. 24. From Marl. Low level tunnel Warcha Mine, Chamber No. 21 between the third and the fourth seam. Coll. B. S. Lamba, 1945.

Fragment No. 10.

Chitinous fragment of an unidentified piece of exoskeleton (Fig. 13) rolled into a loose cylinder and with fine fractures, but without sculpture or setae ; greyish-brown. Length 235 micra.

Slide No. 8. From dolomite from the base of the Saline Series at the junction of Jankush Nulla and Warcha Gorge, Warcha. Coll. B.S. Lamba, 1945.

Fragment No. 11.

Irregular brownish-grey fragment (Fig. 30) of the exoskeleton of an arthropod, without visible sculpture, setae or scars of setae, but irregularly creased and fractured during fossilization. Length 245 micra.

Slide No. 7. From dolomite from the base of the Saline Series at the junction of Jankush Nulla and Warcha Gorge, Warcha. Coll. B.S. Lamba, 1945.

Fragment No 12.

Irregular fragment of a part of an exoskeleton of an arthropod (Fig. 25), with obscure large reticulations on the surface ; no setae or spines anywhere. Length 400 micra.

Slide No. 12. From dolomite from the base of the Saline Series at the junction of Jankush Nulla and Warcha Gorge, Warcha. Coll. B.S. Lamba, 1945.

Fragment No. 13.

Irregular heavily sclerotized and pigmented exoskeletal piece (Fig. 28) with a strongly developed apodeme ; partly folded and overlapping with scars of a few scattered setae, otherwise without sculpture. Length 430 micra.

Slide No. 13. From dolomite from the base of the Saline Series at the junction of Jankush Nulla and Warcha Gorge, Warcha. Coll. B.S. Lamba, 1945.

Fragment No. 14.

Irregular piece of exoskeleton ; faint light brown ; twice as large as maximum width ; folded into two with irregular markings and with a part of vein at one edge (Fig. 26 v). Length 110 micra.

Slide No. 5. From dolomite from the base of the Saline Series at the junction of Jankush Nulla and Warcha Gorge, Warcha. Coll. B.S. Lamba, 1945.

Fragments on Slide No. 17,19,20,21. (Figs. 62-79).

Fragments of exoskeleton of an unidentifiable arthropod, broken and separated while mounting. (Fig. 62-79). Body elongated composed of several segments, nearly cylindrical, covered with stout strong spines arranged in whorls or with scars of fallen setae ; and also with numerous minute hair-like bristles all over ; one stout structure either a mandible (?) or a claw (?) well preserved (Slide No. 21, Fig. 73) about one and a half times as long as broad at the base ; some of the sclerites complete with apical apodemes (Fig 70,74). A few spoon-shaped sensoria of various sizes are scattered irregularly. Spines with rimmed bases inserted into conspicuous pits on the cuticle ; a few of the scars large and conspicuous and with broad rims.

No of fragments on the different slides : Slide No. 17, ten ; No. 19, five ; No. 20, four ; and No. 21, nine.

Slide No. 17,19,20,21. From dolomite from the base of the Saline Series at the junction of Jankush Nulla and Warcha Gorge, Warcha. Coll. B.S. Lamba, 1945.

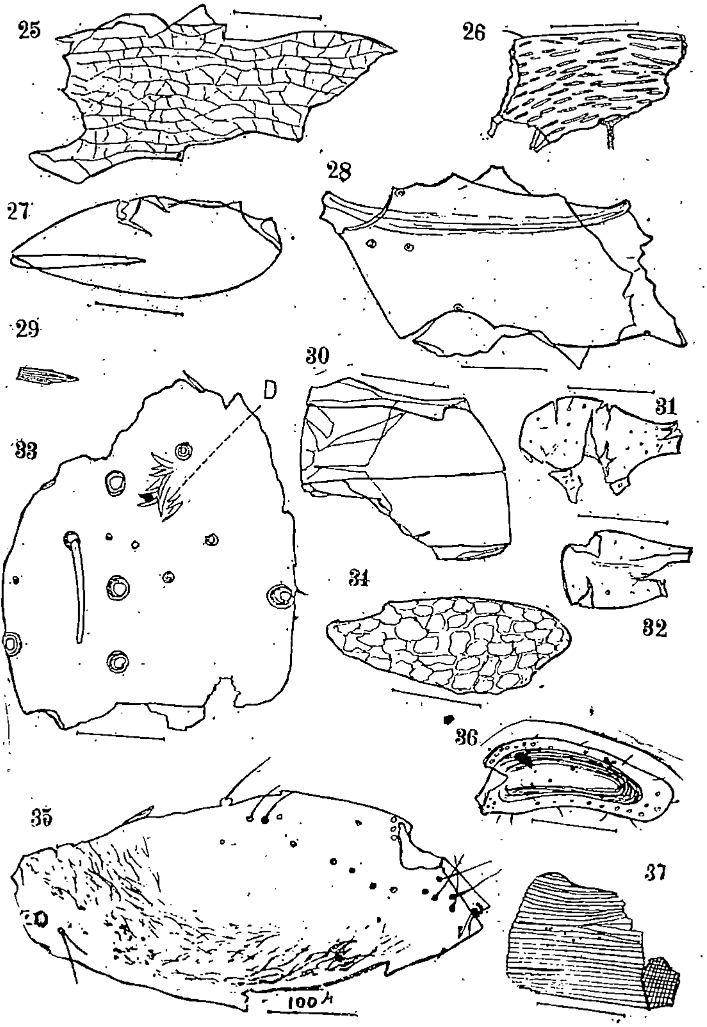

FIG. 25. Fragment of the exoskeleton of an Arthropod.
FIG. 26. Chitinous piece of an Arthropod. V. Vein.
FIG. 27. Empty egg shell of an Acridid.
FIG. 28. Exoskeletal piece of an Arthropod.
FIG. 29. A minute scale, probably of an insect.
FIG. 30. Fragement of the exoskeleton of an Arthropod.
FIG. 31. Knob of halteres of a Nematocerous Diptera.
FIG. 32. Konb of the halteres of a Nematocerous Diptea.
FIG. 33. A highly scelerotized piece of exoskeleton of an Arthropod. D. Denticles.
FIG. 34. Empty egg shell of an Acridid.
FIG. 35. Scape of the antenna of a microhymenopteran.
FIG. 36. Edentate mandibe of an Encyrtid.
FIG. 37. Fragment of a body-scale of a Lepismatid.

MICROFOSSILS FROM NAGPUR

Class INSECTA

Superorder *ORTHOPTEROIDEA*

Order ORTHOPTERA

Family ACRIDIDAE

Frament No. 1.

The empty egg shell of possibly an Acridid, (Fig. 27) very dark brown, coriaceous, smooth ; fractured while handling on the slide but otherwise complete ; eliptic-ovate, nearly two and a half times as long as thick, with undissolved granules of matrix heavily obscuring the interior. Length 923 micra.

Slide No. 1. From Cretaceous limestone clay, Seminar Hills, northwest of the Telankhery Tank, near Village Takli, Nagpur. Coll. M. S. Mani & M. A. Moghe, February, 1950.

Fragment No. 2.

Another specimen of the empty egg shell of the same nature described, (Fig. 34), but somewhat more damaged with the shell partly disintegrated and its inner layer coagulated in patches giving a reticulate appearance. Slightly smaller than the preceding.

Slide No. 25. From Cretaceous Limestone clay ; Seminar Hills, N.W. of Telankhery Tank, near village Takli, Nagpur. Coll. M. S. Mani & M. A. Moghe, Feb., 1950.

Order DERMAPTERA

Fragment No. 3.

The scape and pedicel of an antenna of possibly of a Dermaptera (Figs. 23,24) in fairly good state of preservation ; the scape (Fig. 24) 640 micra long, subfusiform, somewhat abruptly narrowed subapically, about three times as long as thick ; deep yellowish-brown, with scars of a few isolated setae. Pedicel (Fig. 23) obconical, 475 micra long, a little less than three-fourths the length of the scape and three times as long as its own maximum thickness ; basally abruptly narrowed ; the narrowed part a little less than half the thickness at the apex.

Slide No. 50. From Cretaceous Limestone clay ; Seminar

Hills, N. W. of Telankhery Tank ; near Village Takli, Nagpur. Coll. M. S. Mani & M. A. Moghe, Feb., 1950.

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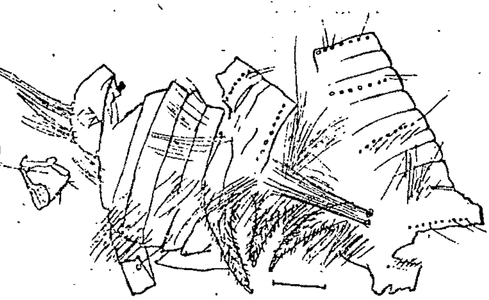

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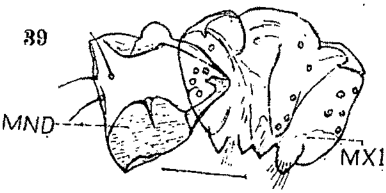

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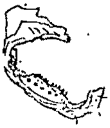

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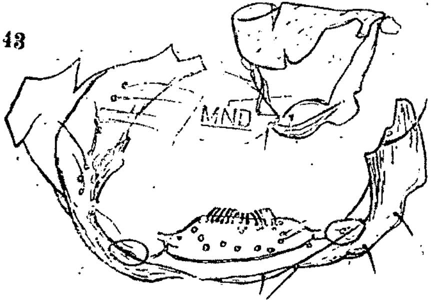

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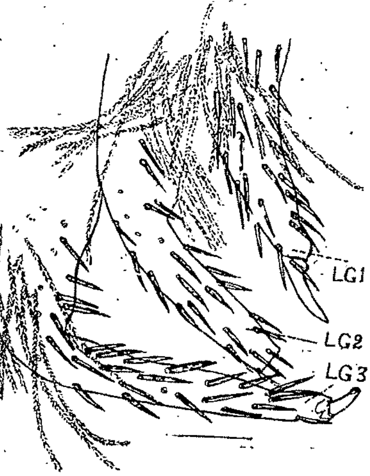

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FIG. 38. Larva of a Dermestid beetle. MND. Mandible.
FIG. 39 & 44. One mandible and maxilla of Dermestid beetle.
FIG. 40 & 43. Head capsule of Dermestid larva.
FIG. 41. Mandible of Dermestid larva.
FIG. 42. Three legs of one side of Dermestid larva. LG1. Foreleg ; LG2. Midleg ; LG3. Hindleg.

Order COLEOPTERA

Suborder *ADEPHAGA*

Family DYTISCIDAE

Fragment No. 4.

Extremely well preserved head capsule and fragments of thorax and abdomen of the larva of a dytiscid beetle (Fig. 1), which it is not possible at present to place in its proper genus. The head is nearly complete, except for the antennae ; roughly triangular with a few isolated slender bristles ; nearly distinct sutures. Labrum (Fig. 1 LBR) large with the anterior margin characteristically dentately produced ; two submedian teeth very large with one small tooth in between and three equally large teeth on either side ; nearly twice as wide as long ; the epipharyngeal surface with fine bristles. The condyle on the cephalic capsule for articulation with the glenoid cavity of the mandible (Fig. 2 MND) large and conspicuous at the sides. Both the mandibles (Fig. 2 MND) in excellent state of preservation, showing the characteristic shape and structure of the predatory dytiscid larvae ; sickle-shaped, more than three times as long as thick at the base ; the mandibular condyle with a diameter a little less than one-seventh the width of the mandible at the base. Maxilla largely damaged and represented by only a part of the maxillary palpus (Fig. 1 MXI) of one side *in situ*, the other detached with the mandible of that side from the body while handling on the slide. Antenna (Fig. 1 ANT) represented by a fragment of the basal segment. The thorax and abdomen crushed and superimposed behind the fragment of the head in a tangled mass of membranes, damaged sclerites and setae. The characteristic head, with the suctorial predatory mandibles and the peculiar labrum readily identify the family. Total length of head 640 micra ; length of mandible 230 micra.

Slide No. 41. From Cretaceous Limestone clay ; Seminar Hills, N. W. of Telankhery Tank ; near village Takli, Nagpur. Coll. M. S. Mani & M.A. Moghe, Feb., 1950.

Family CARABIDAE (?)

Fragment No. 5.

Part of an antenna (Fig. 21) possibly of a Carabid, with two complete flagellar segments (Figs. 21, a & b) and the basal part of the third (Fig. 21 c) yellowish-brown ; densely moderately clothed with elongate, stout, erect, irregular bristles ; the middle segment with a length thrice its diameter, cylindrical ; the other complete segment slightly shorter : subapically these two segments have a regular whorl of somewhat stouter and relatively larger setae arranged in pairs. Total length 960 micra.

Slide No. 42. From Cretaceous Limestone clay ; Seminar Hills : N.W. of Telankhery Tank ; near village Takli, Nagpur. Coll. M. S. Mani & M.A. Moghe, Feb., 1950.

COLEOPTERA incertae sedis

Fragment No. 6.

Terminal part of the abdomen (Fig. 47) of a minute beetle. The fragment comprises the last three tergites, with part of some of the sternites. The tergites have well developed and conspicuous anticostal apodemes (Figs.50,52), which at the sides give off lateral processes. The last tergite bears two groups of closely arranged cuticular sensory setae ; near the posterior margins, the tergite presents a finely reticulated sculpture. The ducts of the gland cells are still preserved in the sclerotized parts. Two obscure, minute stump-like processes (Fig. 48, VGEN), bearing apically a group of setae, probably represent the valvifers of the genital armature. The absenc. of styli and other well developed genital appendages seems to indicate that the fragment is apparently part of the body of Coleoptera.

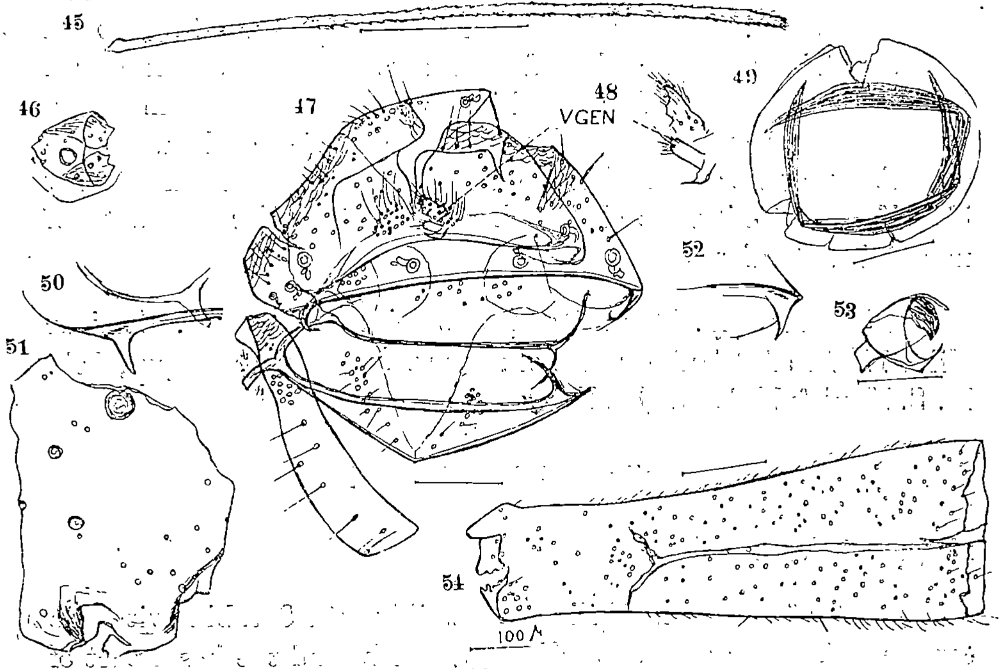


FIG. 45. A single microchaeta of a Dermestid larva.
FIG. 47. Terminal part of the abdomen of a minute beetle. VGEN. Valvifer of the genital armature.
FIG. 51. A Chitnious piece of exoskeleton of an Arthropod.
FIG. 54. Proximal part of tibia of an Arthropod.

Slide No. 49. From Cretaceous Limestone clay, Seminar Hills, N.W. of Telankhery Tank near Village Takli, Nagpur. Coll. M.S. Mani & M.A. Moghe, February, 1950.

Order HYMENOPTERA

Family ICHNEUMONIDAE

Fragment No. 7.

A fragment of the membrane of the posterior and subapical part of the forewing (Fig. 18) in a remarkably excellent state of preservation, with one longitudinal vein and the extreme apical part of the radial vein (Fig. 18^R); discal ciliation and marginal fringe as typical in modern forms; the vein dark-brown. Length 1·2 mm.

Slide No. 40. From Cretaceous Limestone clay, Seminar Hills, N.W. of Telankhery Tank, near Village Takli, Nagpur. Coll. M.S. Mani & M.A. Moghe, February, 1950.

Superfamily CHALCIDOIDEA

Fragment No. 8.

The edentate mandible of a microhymenoptera (Fig. 36), possibly of an Encyrtid Chalcidoid, with the characteristically broadly and obtusely truncated apex and the basal condyle; dark reddish-brown; a little less than three times as long as thick at the base; on the mesial surface with widely separated and isolated crenulations; externally with a few scattered setae. Length 235 micra.

Slide No. 2. From Cretaceous Limestone clay, Seminar Hills, N.W. of Telankhery Tank, near Village Takli, Nagpur. Coll. M.S. Mani & M.A. Moghe, February, 1950.

Fragment No. 9.

A fragment of the scape of the antenna of one of the Microhymenoptera (Fig. 35) showing an obscurely coarsely reticulated sculpture on one side and nearly smooth on the other side; compressed, oval; with a few scattered setae clustered especially towards the tip; the sculpture more conspicuous basally than apically; light yellowish-brown; twice as long as wide; maximum thickness in the middle ; one long seta about the middle nearly twice as long as the others, especially those at the tip. Length 560 micra.

Slide No. 39. From Cretaceous Limestone clay, Seminar Hills, N.W. of Telankhery Tank, near Village Takli, Nagpur. Coll. M. S. Mani & M. A. Moghe, February, 1950.

HYMENOPTERA incertae sedis

Fragment No. 10.

A fragment of the cranial capsule (Fig. 60) bearing one complete antenna (Fig. 60 ANT) with five segments ; elongate slender cylindrical scape, stout conical pedicel, nearly as long as the scape ; two segments of funicle and an elongate oval stout club. The first funicular segment slender, cylindrical, a little less than four times as long as thick and about three times as long as the pedicel. The second funicular segment stout, cordate, nearly equal to the pedicel. Club segment one and one-fourth the times the first funicular segment and a little less than three times its own diameter. The first funicular segment bears a few fine, slender erect setae subapically. Length of antenna 160 micra.

This fragment probably belongs to one of the parasitic group of Hymenoptera like the Chalcids which are characterized by considerable reduction in the antennal segments and their specializalion.

Slide No. 3. From Cretaceous Limestone clay, Seminar Hills, N. W. of Talankhery Tank, near Village Takli, Nagpur. Coll. M. S. Mani & M. A. Moghe, February, 1950.

Order LEPIDOPTERA

Fragment No 11.

Nearly complete flagellum of the antenna of a moth (Fig 56), possibly a Microlepidoptera, still within its pupal sheath ; dark yellowish-brown, about 210 micra long, with numerous transverse short segments. Thickness 19 micra.

Slide No. 15. From Cretaceous Limestone clay, Seminar Hills, N.W. of Telankhery Tank, near Village Takli, Nagpur. Coll. M. S. Mani & M. A. Moghe, February, 1950.

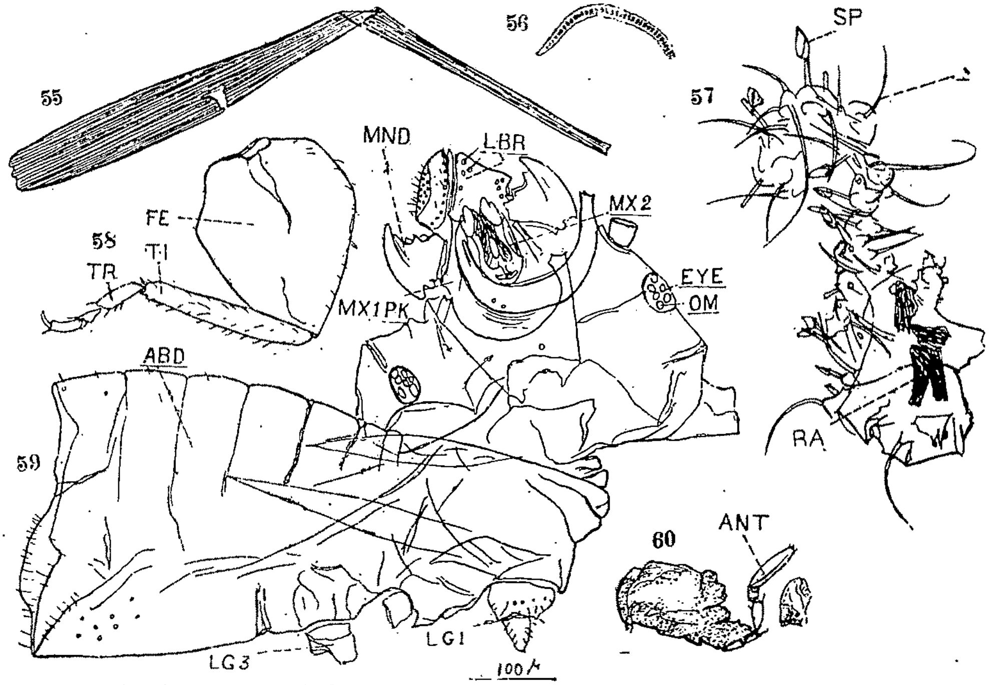


FIG. 55. A complete scale from the wing of a sphingid moth.
FIG. 56. Flagellum of the antenna of a microlepidoptera.
FIG. 57. Damaged exoskeleton of the pupa of a Nematocerous Deptera. RA. Respiratory armature ; S. Spine ; Sp. Spoon-shaped structure.
FIG. 58. Hindleg of a psocid. F. Femur ; TI. Tibia ; TR. Tarsus.
FIG. 59. Head, thorax and part of abdomen of an apterous psocid. ABD. Abdomen ; EYE. Compound eye ; LBR. Labrum ; LGI. Foreleg ; LG3 Hindleg ; MND. Mandible ; MXI PK. Maxillary pick. MX 2. Labium ; O. Ommatidium.
FIG. 60. Cranial capsule of a minute hymenopteran ANT. Antenna.

Order DIPTERA

Suborder NEMATOCERA

Fragment No. 12.

The knob and part of the neck of one of the halteres of a Nematocerous Diptera (Fig. 31) ; moderately large and brownish. The knob two and a half times as long as thick. The short neck about one-third the diameter of the knob, with obscure scars of sensory setae ; partly crushed by the weight of the cover-slip ; the undissolved granules of the matrix still scattered inside. Total length 160 micra.

Slide No. 37. From Cretaceous Limestone clay, Seminar Hills, N.W. of Telankhery Tank, near Village Takli, Nagpur. Coll. M.S. Mani & M.A. Moghe, February, 1950.

Fragment No. 13.

Another example of the knob of the halteres but somewhat more damaged, with just only knob (Fig. 32). Total length 135 micra.

Slide No. 38. From Cretaceous Limestone clay, Seminar Hills, N.W. of Telankhery Tank, near Village Takli, Nagpur. Coll. M.S. Mani & M.A. Moghe, February, 1950.

Six well preserved specimens of the aquatic locomotive pupae of a Nematocerous fly ; three complete and three partially damaged and fragmentary ; among the complete pupae one is smaller than the other two.

Fragment No. 14.

Almost complete exoskeleton of the pupa of a nematocerous fly ; well preserved (Fig. 10). Head and thorax fused with no distinct segmentation and with a few stout long spines ; about three-fourths as long as broad.

Prothoracic respiratory trumpets (Fig. 10RA) well preserved, distinct slightly less than three times as long as thick at the base which is a little less than twice the thickness at the tip ; tip of the trumpet truncate ; the larvel mouth parts well preserved and very close to the respiratory trumpets ; highly sclerotized. Abdomen with only eight visible segments, terminal two missing except the hooked anchoring processes (Fig. 10^{H}) lying loose on one side ; each segment with a conspicuous pair of spoon-shaped structures (Fig. 10^{SP}) arising from a small basal tubercle mid-dorsally on the segment ; the stalk of the spoon equal to the terminal spatula ; the spatula three times as long as wide ; abdomen lighter in colour than the head and thorax.

Body with numerous stout long and curved finely plumose setae (Fig. 10^{S}) arising on small basal tubercles. Length 280 micra.

Slide No. 29. From Cretaceous Limestone clay, Seminar Hills, N. W. of Telankhery Tank, near Village Takli, Nagpur. Coll. M. S. Mani & M.A. Moghe, February, 1950.

Fragment No. 15.

Another similar specimen but larger (Fig. 6) ; exoskeleton complete ; head and the thorax as long as abdomen ; respiratory trumpets well preserved ; three times as long as thick at the base with truncated tips ; head and thorax slightly longer than wide. Abdomen elongated and slightly curved with ten distinct segments with a pair of spoon-shaped structures (Fig 6 ^{SP}) in each segment except the last which carries two paddle-shaped structures (Fig. 6^{P}) ; tip of abdomen with a circlet of numerous acute recurved heavily sclerotized anchoring hooklets (Fig. 6^{H}) ; paddles slightly shorter than spoon-shaped structures. Length 450 micra.

Slide No. 27. From Cretaceous Limestone clay, Seminar Hills, N.W. of Telankhery Tank, near Village Takli, Nagpur. Coll. M.S. Mani & M.A. Moghe, February, 1950.

Fragment No. 16.

Another complete exoskeleton of a similar pupa. Abdomen almost straight and slightly longer than head and thorax combined; head and thorax well preserved with the respiratory trumpets (Fig. 14 ^{RA}) and the larval mouth parts ; trumpets nearly three times as long as thick at the base ; tip truncated three-fourths the thickness at the base. Abdomen with ten segments, spoon-shaped structure missing on the 9th segment ; last abdominal segment with two paddle-shaped structures (Fig. 14 ^{P}) and circlet of heavily sclerotized recurved hooks. Length 445 micra.

Slide No. 34. From Cretaceous Limestone clay. Seminar Hills, N.W. of Telankhery Tank, near Village Takli, Nagpur. Coll. M.S. Mani & M.A. Moghe, February, 1950.

Fragment No. 17.

Incomplete and practically damaged exoskeleton of the pupa of a Nematocerous Diptera (Fig. 7) head and thorax disintegrated and unrecognizable except for the respiratory trumpets (Fig. 7$^{\text{RA}}$) and the larval mouth parts; abdomen incomplete; a few spines (Fig. 7$^{\text{S}}$) and spoon-shaped structures (Fig. 7$^{\text{SP}}$) intact. Length 300 micra.

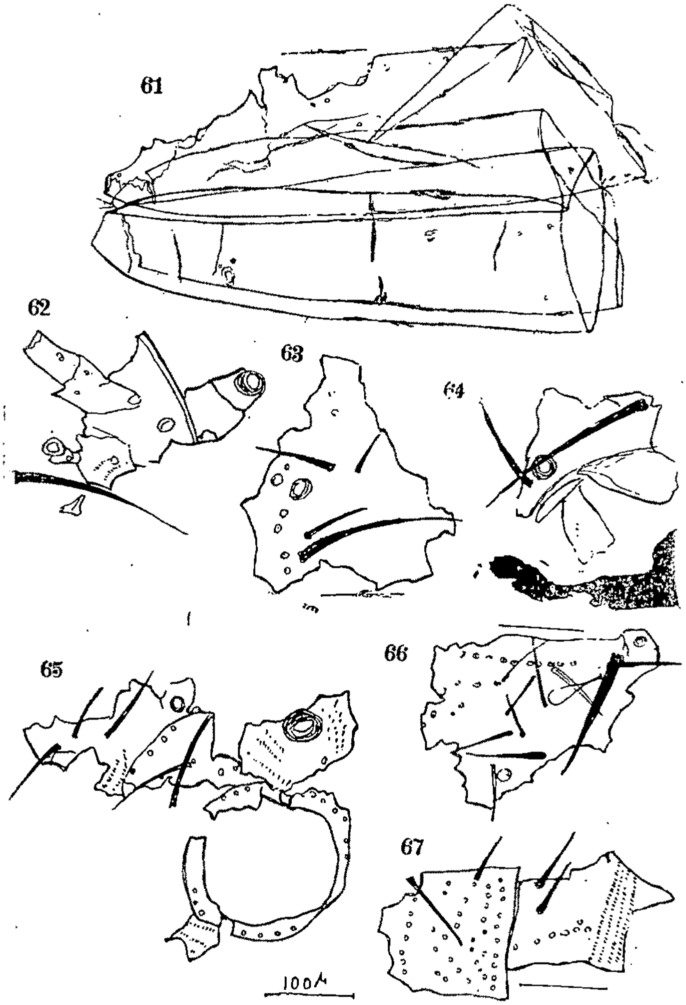


FIG. 61. Two emarginate chitinous fragments of the exoskeleton of an Arthropod.
FIG. 62-67. Broken pieces of the exoskeleton of an unidentified Arthropod.

Slide No. 28. From Cretaceous Limestone clay, Seminar Hills, N.W. of Telankhery Tank, near village Takli, Nagpur. Coll. M.S. Mani & M.A. Moghe, February, 1950.

Fragment No. 18.

Slightly damaged exoskeleton of the pupa of a Nematocerous

Diptera (Fig. 57); head and thorax not preserved fully but the respiratory trumpets (Fig. 57 ^{RA}) and larval mouth parts preserved; abdomen complete and well preserved except for the tip; with spoon shaped structures, more or less intact. Length 325 micra.

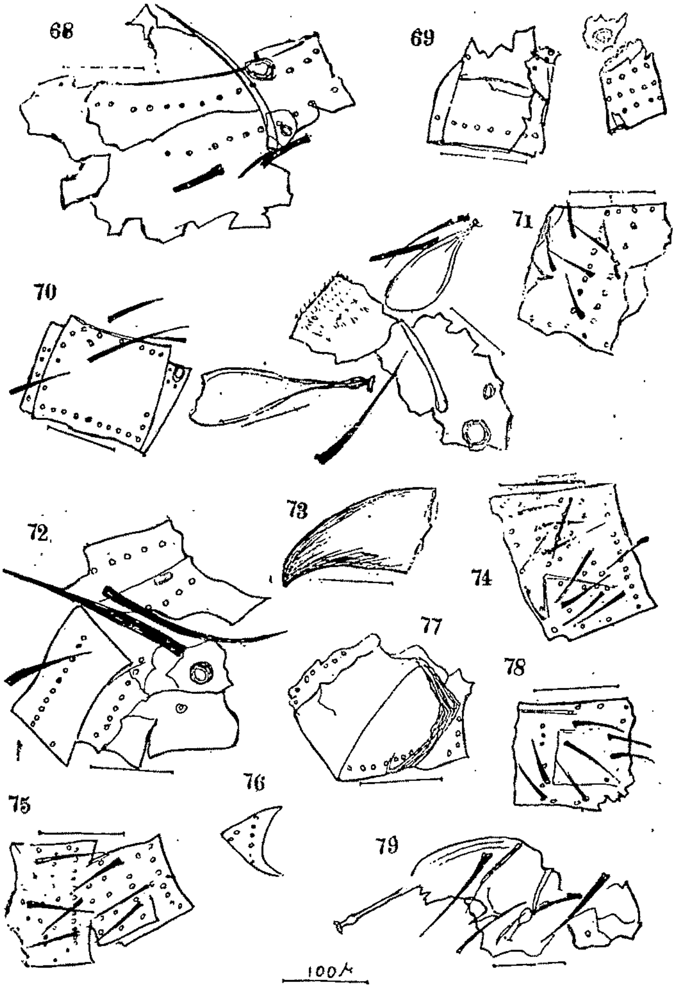


FIG. 68-79. Broken pieces of the exoskeleton of an unidentified Arthropod.

Slide No. 26. From Cretaceous Limestone clay, Seminar Hills, N.W. of Telankhery Tank, near Village Takli, Nagpur. Coll. M.S. Mani & M.A. Moghe, February, 1950.

Fragment No. 19.

A small fragment of the exoskeleton of the pupa of a Nematocerous Diptera (Fig. 15); the respiratory trumpets (Fig. 15 ^{RA}) and larval mouth parts and a very small part of the abdomen are preserved. Abdomen incomplete ; practically broken and damaged; spines (Fig. 15 ^{S}) only a few ; spoon-shaped structures (Fig. 15 ^{SP}) almost all broken off. Length 215 micra.

Slide No. 32. From Cretaceous Limestone clay, Seminar Hills, N.W. of Telankhery Tank, near Village Takli, Nagpur. Coll. M.S. Mani & M.A. Moghe, February, 1950.

Order THYSANURA

Family Lepismatidae

Fragment No. 20.

Broken fragment of a scale (Fig. 37) from the body of a Lepismatid ; hyaline, partly folded at one end ; 240 micra long, with fine longitudinal striations about 2,600 per mm.

Slide No. 39. From Cretaceous Limestone clay, Seminar Hills, N.W. of Telankhery Tank, near Village Takli, Nagpur. Coll. M.S. Mani & M.A. Moghe, February, 1950.

INCERTAE SEDIS

Fragment No. 21.

Partly damaged and incompletely preserved piece of the compound eye of an arthropod (Fig. 22) showing the regularly arranged ommatidia but distorted due to bad preservation ; about one and half times as long as broad and with part of the optic lobe adhering at the base ; the ommatidia are arranged parallel to one another, with pigmentation more concentrated at the base in each ommatidium. The concentration of the pigments towards the base suggests that the eye must have been that of a nocturnal or crepescular insect.

Slide No. 14. From Cretaceous Limestone clay, Seminar Hills, N.W. of Telankhery Tank, near Village Takli, Nagpur. Coll. M.S. Mani & M.A. Moghe, February, 1950.

Fragment No. 22.

An unidentifiable piece of an arthropod (Fig. 33); heavily sclerotized, dark-yellowish-brown; slightly wider than it is long; with large conspicuous spine and six or seven large scars of fallen setae. Towards one end is a group of minute irregularly set denticle-like structures (Fig. 33 ^{D}); each denticle three times as long as broad at the base; slightly bent and sabre-shaped.

Overlapping the specimen is a minute scale-like structure probably of an insect (Fig. 29); about five times as long as its maximum width, with very fine longitudinal striations.

Slide No. 4. From Cretaceous Limestone clay, Seminar Hills, N.W. of Telankhery Tank, near Village Takli, Nagpur. Coll. M.S. Mani & M.A. Moghe, February, 1950.

Fragment No. 23.

An irregularly subtriangular piece of an arthropod exoskeleton (Fig. 3) with a few long, slender, aciculate sensory setae but showing no visible sculpture. Length 325 micra.

Slide No. 11. From Cretaceous Limestone clay, Seminar Hills, N.W. of Telankhery Tank, near Village Takli, Nagpur. Coll. M.S. Mani & M.A. Moghe, February, 1950.

Fragment No. 24.

The proximal part of the tibia of an unidentified arthropod (Fig. 54); cylindrical; very dark reddish-brown, with the proximal articular processes of the knee-joint; very fine setae scattered all over the surface. Proximally the fragment is slender but becoming stouter gradually towards the distal end. The length of the fragment is five times the thickness at the base, a little over three times the thickness at broken tip. Total length 645 micra.

Slide No. 35. From Cretaceous Limestone clay, Seminar Hills, N.W. of Telankhery Tank, near Village Takli, Nagpur. Coll. M.S. Mani & M.A. Moghe, February, 1950.

Fragment No. 25.

Irregular piece of exoskeleton of an arthropod (Fig. 51); very dark brown and heavily cuticularized especially at one end, without sculpture, but with three scars of stout setae and one or two smaller ones. Length 400 micra.

Slide No. 30. From Cretaceous Limestone clay, Seminar Hills, N.W. of Telankhery Tank, near Village Takli, Nagpur. Coll. M.S. Mani & M.A. Moghe, February, 1950.

Fragment No. 26.

Empty egg shell of an unidentifiable arthropod (Fig. 19), slightly damaged while handling on the slide ; spherical dark brown and minutely sculptured with circular uniform moderately deep pits. The periphery of the shell crenulated.

Slide No. 6. From Cretaceous Limestone clay, Seminar Hills, N.W. of Telankhery Tank, near Village Takli, Nagpur. Coll. M S. Mani & M.A. Moghe, February, 1950.

Fragment No. 27.

A slightly better preserved specimen than the preceding one (Fig. 4), with a thin transparent membrane attached, though not actually touching the shell.

Slide No. 31. From Cretaceous Limestone clay, Seminar Hills, N.W. of Telankhery Tank, near Village Takli, Nagpur. Coll. M.S. Mani & M.A. Moghe, February, 1950.

Fragment No. 28.

Another specimen of the empty egg shell of an unidentifiable arthropod (Fig. 5) ; slightly damaged ; paler than the preceding ones.

Slide No. 47. From Cretaceous Limestone clay, Seminar Hills, N.W. of Telankhery Tank, near Village Takli, Nagpur. Coll. M.S. Mani & M.A. Moghe, February, 1950.

BIOSYNTHESIS OF VITAMINS

By H. N. De, M.Sc., Ph.D., *Reader in Biochemistry, Physiology Department, Mahatma Gandhi Memorial Medical College, Indore (M. B.).*

The process of symbiosis is an universal phenomenon encountered in the everyday process of life of all the organisms, whether lower or higher. The occurrence of certain species of bacteria of the *Rhizobium*-group in the nodules of leguminous plants and their intimate association with them is a typical example of such phenomena of symbiosis, in which the plant and the bacteria, living side by side, mutually help each other—the plant supplies the necessary carbohydrate to the bacteria for their proper growth, whereas the bacteria supply or fix the atmospheric nitrogen to the plant for its growth. There are certain cases in which the bacteria by their neighbouring behaviour with other microflora help their growth mutually by the above process of symbiosis.

A host of different species of bacteria reside in the human intestine. According to the view of the previous workers (61, 12) these were merely regarded rather as the source for the waste of the food protein in the form of bacterial body protein excreted in the faeces. That these bacteria also symbiotically help the human body was realized only in recent years. Although they reside permanently in the intestine of the human body and feed themselves at the expense of food nutrients, they synthesize a large amount of different vitamins, which are also available to the human body for metabolic processes. A new concept has thus been postulated in explaining the existence of bacteria in the human intestine on the basis of their capacity for the "Biosynthesis of Vitamins."

The historical basis of symbiosis by the intestinal bacteria dates back to 1911, when Osborne Mendel (63) noted that the rats on certain purified diets declined in weight and became coprophagous and that this decline in weight could be arrested by incorporating in their diet the faeces from the normal animal. Sterilisation of the faeces although reduced the above beneficial effect, did not totally abolish it, thus indicating that some thermostable factor was concerned in the process in addition to either the living organism or the thermolabile factor. It was therefore suspected that some growth factor was elaborated by the bacteria of the faeces, which could maintain growth of the rats on deficient diet.

Indirect proof that some growth factor was produced in the faeces by the bacteria came first from Theiler and his associates (84). They fed cattle on experimental diet deficient in vitamin B_1 but did not find any evidence of deficiency in this animal, whereas the pigeons fed on the same diet developed polyneuritis promptly. They assumed that either the vitamin requirement of cattle was extremely low or it had been satisfied by bacterial synthesis in the lumen. The demonstration by Guerrant and his collaborators (35) that the faeces of the caecectomised rats possess diminished power to protect against vitamin B-deficiencies also gave the direct evidence that the vitamins are probably synthesized by the microflora of the intestine. By *in vitro* culture experiments in the synthetic medium, Pacini and Russel (64) first proved that the typhoid bacilli contributed to the medium a factor, which when fed to the rats stimulated their rate of growth. Gradually researches in this line revealed that different microorganisms possess the capacity to synthesize a large amount of vitamins, specially of the B-group, vitamins E and K. The direct evidence, that the intestinal microflora are also engaged in the large-scale synthesis of different vitamins and that vitamins are actually utilised by the host animal, has been obtained only after the discovery of the highly bacteriostatic agents like sulpha drugs, which have been used as the tool for tracing the bacterial synthesis of the vitamins in the intestine.

SULPHA DRUGS AS THE NEW TOOL FOR TRACING THE BIOSYNTHESIS

The announcement by Marshall *et al.* (51) that a new sulpha drug—sulphaguanidine—because of its poor absorption and long duration in the intestine, is an effective bacteriostatic agent for the intestinal microorganisms, encouraged many nutrition experts for the study of the microbial synthesis of vitamins in the intestine by the use of this new tool. Gradually more potent and poorly absorbed sulphonamides like sulphasuxidine (succinylsulphathiazole), pthalylsulphathiazole, etc., have been discovered and have also been used in the above investigations.

The real assessment of the biosynthesis of vitamins by the use of the above drugs has been achieved by observations based on two lines :— (1) by the induction of the avitaminosis by partial sterilisation of the intestine by the above sulpha drugs, followed by the cure of the avitaminosis by giving adequate dose of appropriate vitamins and (2) by observation of the decreased urinary and faecal elimination of a particular vitamin under the influence of these drugs when these are incorporated with the normal diet.

This technique has been applied both in cases of ruminants and non-ruminants to trace out the microbial synthesis but in the present review we will confine ourselves only to the work on non-ruminants.

Vitamin K.—The first reported use of sulpha drug in tracing out the microbial synthesis of vitamin K in the intestine is available from Black *et al.* (4, 5), who noted failure in growth, haemorrhages and hypoprothombinemia in rats given sulphaguanidine along with the purified diet and this has been supported by the observations of other workers as Daft *et al.* (14) and Kornberg *et al.* (44). The latter group of workers not only used the poorly absorbed sulpha durgs like sulphaguanidine, etc., but also used the highly absorbed ones as sulphadiazine, sulphapyrazine, sulphathiazole, etc. It is evident from their investigation that not only the poorly absorbed sulpha drugs can precipitate the deficiencies because of their longer period of duration in contact with the microflora but also the soluble or highly absorbed ones can do so inspite of their short duration in the intestines.

Folic acid.—The evidence of the biosynthesis of folic acid gradually accumulated in the literature from the observations of the different workers as Spies *et al.* (81), Axelrod *et al.* (1), Kornberg *et al.* (43), Daft *et al.* (15) and others that serious growth disturbances and severe blood dyscrasias (involving abnormalities of various white blood cells and of bone marrow) produced in rats feeding sulphaguanidine could be cured by giving liver extract or folic acid.

Thiamine.—The first report as to the synthesis of thiamine in the intestine by microflora came from Guerrant and Dutcher (36), who showed that the rat faeces contained sufficient amount of thiamine, thus indicating the possibility of its synthesis in the intestine. Najjar and Holt (56) found considerable amounts of thiamine in the human stool. By feeding sulpha drug, they conclusively proved that the intestinal microflora are engaged in the synthesis of this vitamin, which is actually available to the host for metabolic processes. De, Malaker and Paul (24) by using the sulpha drug—sulphadimethylpyrimidine—have noted large-scale synthesis of thiamine by the intestinal microflora.

Nicotinic acid.—Previous reports show that the rats thrive well on black-tongue-producing diet. Balance studies with this diet revealed that the excretion of nicotinic acid was much higher than the intake—the faecal being 4 to 5 times more than the intake (41), thus indicating the possibility of large-scale synthesis of nicotinic acid by intestinal microflora. Later on, other workers (72, 31) by the use of sulpha drug have conclusively proved the microbial synthesis of nicotinic acid in the rat intestine. Evidence of the synthesis in the human intestine came gradually from the works of Ellinger *et al.* (28-31) and Benesch (3), who showed that the intake of succinyl-sulphathiazole and others produced a great decrease not only of nicotinic acid but also of N^1-methylnicotinamide excretion in the urine, showing that nicotinic acid is synthesized by the microflora also in the form of nicotinamide. De, Malaker and Paul (*loc. cit.*), in their studies on the biosynthesis of nicotinic acid in the human intestine, have also determined the daily synthesis of this vitamin in the intestine under normal dietary conditions and calculated this to be between 9 to 10 mg.

Riboflavin—Guerrant and Dutcher (36) first conceived that the rat can synthesize riboflavin with the help of microflora in the intestine. Conclusive evidence came later on from different workers as Schweigert *et al.* (72), Skeggs and Wright (77), Czeczkes and Guggenheim (13) and others who traced out the biosynthesis of this vitamin by the intestinal microflora by using the sulpha drugs technique. De and Roy (26, 27) also studied the above biosynthesis in rat intestine by using different sulpha drugs.

There are only a few reports regarding the synthesis of this growth factor in the human intestine—the only conclusive evidence being available from the work of Najjar *et al.* (57). It is evident from his work that the amount of riboflavin synthesized is much greater than that of thiamine.

Biotin, pantothenic acid, etc.—The biosynthesis of biotin and pantothenic acid and other members of B-complex has been traced out by observing the deficiency syndromes of these vitamins in rats after incorporation of sulphasauxidine, etc., in the normal diet (58, 87, 88).

Vitamin E.—The incorporation of sulpha drugs in the diet has been found to precipitate the deficiency symptoms of vitamin E (16). By *in vitro* cultures it has been noted that vitamin E is not synthesized by the intestinal microflora but the above observations have been explained on the basis of recent announcement by Milhorat and Bartels (52) that the biologically effective form of tocopherol is a complex compound with inositol and the sulpha drug ingestion inhibits the biosynthesis of inositol, thus leading indirectly to the deficiencies of vitamin E.

Literature on the biosynthesis of different other vitamins is enormous but it is not possible to make a review of this within present limits.

MICROORGANISMS INVOLVED IN THE BIOSYNTHESIS

All the different intestinal microflora present in normal human adult as *Coliform bacilli*, *Staph. aureus*, *Staph. albus*, *B. proteus, Enterococci, Ps. pyocyanea, B. aerogens, Bact. coli communis; Cl. welchi,* etc., are engaged in the biosynthesis of different vitamins. Burkholder and McVeigh (10) have shown by studies in pure culture medium that six typical flora of the intestine as *Bact. aerogens,* etc., can synthesize a considerable amount of thiamine, riboflavin, nicotinic acid, biotin and vitamin K. The amount of synthesis of a particular vitamin depends however on the nature of the microorganisms.

There are certain flora which can also synthesize the coenzymes I and II from nicotinamide, and cocarboxylase from thiamine and can supply the host animal with the preformed coenzyme components of the different dehydrogenase and carboxylase systems.

The value of this synthesized vitamin depends on as to how much quantity of the synthesized amount is actually available to the host body. A certain amount of the total synthesis is actually required for the normal metabolic processes of the microflora and the rest is available to the host as a part of the total daily requirement. Mitchell and Isbell (53) of the University of Texas, demonstrated by autopsy studies in rats that folic acid, biotin and pantothenic acid are released by the caecal bacteria and absorbed by the caecal wall. The vitamin concentration in the wall of the lower intestine is much lower than in the caecum, thus showing that most of the synthesized vitamins are absorbed through caecum. The results of these experiments are also substantiated by the *in vitro* studies by Burkholder and McVeigh (*loc. cit.*), who found that in cultures of *B. aerogens* for 111 hours, about 30% of biotin, thiamine, and riboflavin, and about 40% of nicotinic acid produced by the bacteria were present in the filtrate. In another report on the synthesis of vitamins in pure culture by *Bact. aerogens, B. prodigiosus, Ps. fluorescens, Proteus vulgaris* and *Cl. butyricuo,* Thomson (83) revealed that with all the eight B-vitamins studied, only a certain amount of the vitamin required for the activities of the cell is retained by the microorganisms, the excess is excreted in the medium. Moreover the amount of vitamins in the cells showed a remarkable constancy in all the species investigated, whereas the amount outside the cell was much more variable. Thus there were two and a half times as much riboflavin, one and a half times as much nicotinic acid and twelve times as much of biotin in the medium of aerobic cultures of *Bact. aerogens* as in the cells of intestinal microorganisms. The possibility of autolysis of the cells for such huge release of the vitamins in the medium was further studied by the same author with the culture of *Proteus vulgaris* and it was shown that the biotin release paralleled the growth of the bacteria and thus the above possibility of the autolysis for large liberation in the medium was ruled out. In contrast with this observation, the work of Najjar and Barrett (55) on biosynthesis of riboflavin by *B. coli communis* in the synthetic medium shows that most of the synthesized riboflavin is held within the bacterial cells and the liberation into the medium occurs only after their disintegration. De and Datta Jr. (*loc. cit.*) and De & Roy (26) in their studies on the biosynthesis of nicotinic acid and riboflavin in the rat intestine have viewed that major amount of synthesized nicotinic acid is liberated in the medium and so utilized by the host animal, as was evidenced from larger excretion in the urine than in faeces, whereas in case of riboflavin most of the synthesized vitamin is held within the cell bodies and so excreted largely in the faeces.

EQUILIBRIUM BETWEEN SYNTHESIS AND UTILISATION BY THE INTESTINAL MICROFLORA

In most of the above work the biosynthesis has been assessed by observing the decreased elimination of a particular vitamin in the urine and faeces under the influence of the sulpha drug but some cases are reported, in which the elimination of the vitamin was rather increased under the influence of the sulpha drug. Najjar *et al.* (57) observed that feeding of succinylsulphathiazole to human subjects increased their riboflavin output but with simultaneous decrease in the output of thiamine. De, Malker and Paul (24) and De, Roy and Datta (20) by their studies on effect of different sulpha drugs and other bacteriostatic agents on the biosynthesis of B-vitamins have observed that the sulpha drugs such as sulphapyridine, sulphaguanidine, sulphathiazole and pthalylsulphathiazole increased the elimination of thiamine, riboflavin and nicotinic acid in human subjects and in rats to a considerable extent, whereas the sulpha drugs of the pyrimidine group as sulphadimethylpyrimidine and sulphadiazine decreased the elimination of thiamine but increased the eliminations of nicotinic acid and riboflavin. The possibility of interference of these sulpha drugs with the absorption and utilisation of these vitamins in causing such increased elimination was also studied by employing new technique of the test dose experiment and it has been found that these and other drugs do not actually interfere with the absorption and utilisation of the above vitamins.

The above peculiar behaviour of the sulpha drugs of pyrimidine group in causing increased elimination of nicotinic acid and riboflavin and simultaneous decreased elimination of thiamine has been explained on the basis of the presence of two opposing strains of bacteria in equilibrium—those which synthesize thiamine but require nicotinic acid and riboflavin as their growth factors and those which synthesize nicotinic acid and riboflavin alone or separately—and probably the above sulpha drugs, because of the presence of pyrimidine group common to thiamine interferes with the synthesis of thiamine by the thiamine-synthesizing microflora and so a large amount of nicotinic acid and riboflavin which would, otherwise, have been utilized by these microflora is thereby spared for utilization by the host animal and producing higher excretion in urine and faeces. Although theory of Fildes (33), as modified by Nimmo-Smith *et al.* (60), suggests that the bacteriostatic action of sulphonamides is elaborated by its interference with the utilization of p-aminobenzoic acid in the synthesis of folic acid—the essential metabolite for all the microflora—but the possibility that the sulphonamides also elaborate their bacteriostatic action through other structural configurations as the thiazole group, pyridine group, etc., in the synthesis of thiamine, nicotinic acid, etc., has been suggested by Welch (89) and others after the observations by different workers as Feldt (32), Hawking (38), Tamura (82), Welch (86) and others that in some cases the p-aminobenzoic acid could not counteract the bacteriostatic effect of sulpha drugs.

That an equilibrium actually exists between the microflora which synthesize one growth factor and those which utilize this for their growth has been observed by Benesch (3), who showed by *in vitro* cultures of the faecial bacteria that certain aerobic organisms synthesize nicotinic acid, whereas the anaerobic ones actually utilize it for their growth. There are many other examples of such symbiosis between the microorganisms, in which the vitamin synthesized by one or more species act as essential nutrient for the others inhabiting in the same medium.

DIETARY CONSTITUENTS AS THE INFLUENCING FACTORS

Since the intestinal synthesis of the vitamins are performed by the microflora residing there, it was thought that this biosynthetic process might be affected by their growth in the intestine *i.e.*, by the change in the constituents of the medium. Work has, therefore, been started on this line to see how far the different food constituents as carbohydrates, proteins, etc., in the medium affect the above process. The effect of different carbohydrate components on such process have been studied extensively but the results are divergent.

Guerrant and Dutcher (35) by feeding different types of carbohydrates in rats have shown that dextrinised corn starch produced greater synthesis of thiamine and riboflavin in the intestine than the undextrinised starch, sucrose, glucose or lactose. Najjar and Holt (56) by conducting human experiments have shown that dextrimaltose is much superior to sucrose when that is used as the sole source of carbohydrate of the diet in promoting the intestinal synthesis of thiamine—glucose producing an effect intermediate between the above two extremes. Schweigert *et al.* (76), Skeggs and Wright (77) by further experiments on rats have shown that lactose is not inferior but rather ranks in same line as maltose in promoting the biosynthesis of riboflavin. While reviewing the position of glucose in this respect it seems likely that it is inferior to lactose (76) but superior to starch and cellulose (77). Some work has also been done to study the effect of carbohydrates on the biosynthesis of nicotinic acid. In their investigations on the effect of carbohydrates on the pellagragenic role of corn, Schweigert *et al.* (76) and Krehl (45, 46, 47) showed that dextrin, starch and glucose are much superior to sucrose in protecting the deleterious effect of corn. Schweigert and Pearson (74) showed that cerelose is much superior to sucrose in producing better growth and more excretion of nicotinic acid and its metabolic products.

In most of the above work the diets used were not made free from the vitamin under investigation—a condition which is of primary importance in order to have precise knowledge of the actual amount of vitamin synthesized in the body as influenced by the dietary constituents—and so the observations by the above workers do not present conclusive evidence of the above aspect of biosynthesis. This point has been further studied by De and his collaborators (19, 26) by feeding the young and adult rats on diets completely freed from niacin and riboflavin, with and without the supplement of sulpha drug, and by determining the excretion and the liver and kidney contents of these vitamins. After a prolonged period under above dietary conditions it has been conclusively proved that out of lactose, dextrin, starch, glucose and sucrose used in the diets, lactose produced the maximum biosynthesis of niacin and riboflavin and sucrose the minimum—the other carbohydrates occupying the intermediate position in this respect. It has further been observed that the young rats possess greater capacity than the adult ones for the biosynthesis of the above two vitamins in the intestine.

Precursors for the biosynthesis.—The participation of the intestinal bacteria in the process of synthesis of vitamins in the intestine having been established, the attention of the nutrition workers was then directed to trace out the precursors for the above synthesis of different vitamins. As a result of this a completely new field of researches in the line of biosynthetic mechanism has been developed only in the last few years.

Precursors of nicotinic acid.—No vitamin has received greater attention than nicotinic acid regarding the precursors of its biosynthesis.

Previous observations by different workers that certain proteins such as casein increase the biosynthesis of vitamin led to the belief that the process is in some way linked with the amino acid metabolism and being prompted by this thought Guggenheim (37) in 1940 suggested a scheme for nicotinic acid synthesis, based on the amino acid-ornithine—as the precursor and with proline and d-amino valeric acid as the intermediates—and the scheme is represented as follows: —

Ornithine→Proline→d-amino valeric acid→d-pentenic acid→guavicin→Nicotinic acid ....

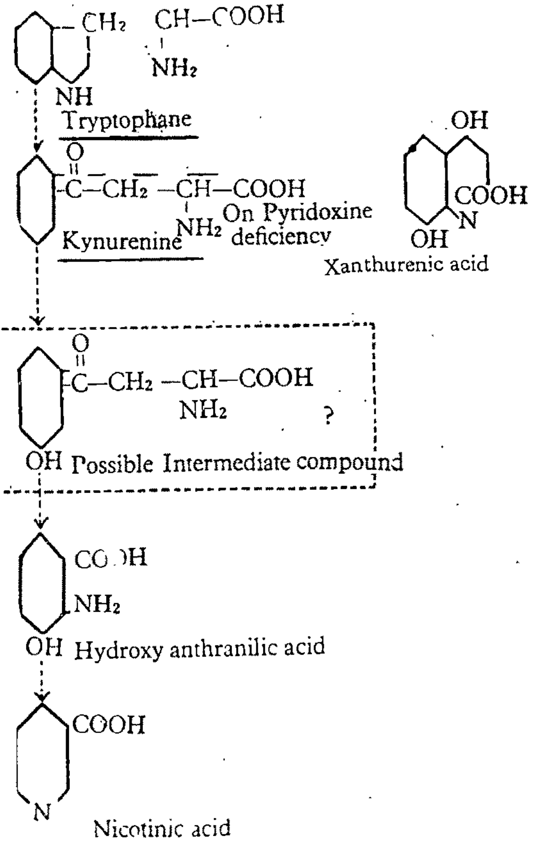
The author and his collaborator (19) made attempts to test the validity of this scheme by feeding the amino acid arginine—the precursor of ornithine—and also proline to the rats and it has been observed that these two amino acids when given with the nitrogen-free and nicotinic-acid-free diet do not produce any change in the nicotinic acid excretion of the animals thus pointing out conclusively that the above scheme of Guggenheim does not hold good in case of the animals, although it may be successful in case of plants, as has been observed by some workers. Ellinger and Kader (31) also failed to find any response in the production of nicotinic acid in rats by the above amino acids, although the bacteria isolated from the rat faeces successfully produced the above vitamin from these amino acids when cultured in the medium.

The observations by Krehl *et al.* (45) showed that the deleterious effect of corn in retarding the growth and in developing the symptoms of pellagra could be rectified by inclusion in the diet either of nicotinic acid or protein rich in tryptophane or tryptophane alone and thus a new precursor, tryptophane, has been brought to light for the biosynthesis of nicotinic acid in the animal body. This tryptophane-nicotinic acid relationship has further been studied by other workers (66, 73, 31) on animals and also on human subjects (68, 65). A great deal of speculation was raised as regards the site of conversion of tryptophane to nicotinic acid and most of the above workers were of the opinion that this conversion takes place in the intestine by the microflora residing therein.

The author and his collaborators (19) by feeding tryptophane with and without sulphaguanidine have clearly shown that although the main site of conversion of tryptophane to nicotinic acid is the intestine with the help of microflora, the possibility of involvement of some other tissues as well cannot be ruled out. This has been confirmed by their further experiments with isolated *in vitro* tissue culture experiments (21), in which it has been definitely proved that the liver and the intestine possess the capacity to convert tryptophane to nicotinic acid when incubated with this amino acid. This investigation has clarified the doubt regarding the sites of conversion of tryptophane to nicotinic acid in the body.

Then the question was raised regarding the possible intermediates in the above pathway of conversion. In course of studies on the synthesis of nicotinic acid from tryptophane by certain mutants of *Neurospora,* Bonner and Beadle (6) and Beadle *et al.* (2) observed that the medium contained not only nicotinic acid but also kynurenine and hydroxy-anthranilic acid. Moreover kynurenine or hydroxy-anthranilic acid, when added in the medium, are easily converted to nicotinic acid (54,7). So the above two compounds were regarded as the intermediates in the conversion of trytophane to nicotinic acid and a scheme was suggested for such conversion. This scheme has also proved to be valid when applied in case of rats and mammals as a whole and it has been found that kynurenine and hydroxy-anthranilic acid can replace tryptophane in the diet for the synthesis of nicotinic acid in the body (54, 39, 40).

While studying the effect of other vitamins on the biosynthesis of nicotinic acid from tryptophane, it has been further observed that pyridoxine plays a very important role in this process and the rats on diet deficient with regard to this vitamin produces less excretion of nicotinic acid and its derivatives in the urine (73, 42, 67). The observations of Cartwright *et al.* (11) and Rosen *et al.* (67) that pyridoxine deficiency produces a large increase of the excretion of kynurenine and its oxidation product xanthurenic acid with simultaneous decrease of the excretion of nicotinic acid suggest that pyridoxine elaborates its action in the process of tryptophane-nicotinic acid conversion at that stage where the intermediate kynurenine instead of undergoing oxidation to xanthurenic acid is converted to hydroxy-anthranilic acid by oxidative deamination, which is further oxidised and give rise to nicotinic acid.



The role of pyridoxine in nutrition has been realised only in recent years and it has been revealed by different workers (78, 79, 80, 71, 49, 34, 85, 70, 62), that this vitamin acts as a coenzyme in the transaminase system involved in the transamination of amino acids and the action is elaborated by the reversible reaction of pyridoxal$\rightleftharpoons$pyridoxamine by which the keto acids are converted to amino acids by the transfer of the amino group from pyridoxamine and vice versa. On the basis of these observations it may be postulated that pyridoxine plays its role in the process of conversion of tryptophane to nicotinic acid by entering in the transaminase system in the form of pyridoxal phosphate in the deamination and transamination of the reversible reaction kynurenine$\rightleftharpoons$hydroxyanthranilic acid—an important step in the above process of tryptophane-nicotinic conversion. The whole scheme of reactions is represented in the figure on page 121.

Other precursors of nicotinic acid.—In the laboratory, nicotinic acid can be prepared by oxidation of nicotine, quinoline, 3-3' dipyridyl or *B*-picoline. These were fed to rats to see whether the pyridine derivatives can serve as the source of nicotinic acid synthesis in the animal body (18) and it has been found that except *B*-picoline none of the above compounds can stimulate the biosynthesis of nicotinic acid in the animals. This also shows that the animal body cannot perform the drastic oxidation reactions as are necessary for the conversion of the nicotine, quinoline or 3-3' dipyridyl to nicotinic acid but it can only oxidise those pyridine derivatives which are substituted at the *B*-position of the ring by the methyl group, as in case of *B*-picoline—the oxidation taking place only at the side chain.

In the laboratory, Bovarnick (9) synthesized nicotinamide by heating asparagine with any of the amino acids—glutanic acid, methionine, ornithine or proline. Experiments were, therefore, performed by feeding asparagine and methionine to the animals to see whether the animals can perform similar chemical reactions as are required for laboratory synthesis of nicotinic acid or its amide and it has been found by De and Guha (21, 22) that the animals actually cannot do so.

Not only the organic nitrogenous compounds as tryptophane, *B*-picoline, etc., but also the inorganic compound like ammonium sulphate has been found to stimulate the biosynthesis of nicotinic acid when fed to animals (21, 22). Potassium nitrate failed to do so and this suggests the animal body can utilise the ammonium ion and not the nitrate ion *i.e.*, the body or the intestinal microflora lack in the capacity for reducing the nitrate ion like the soil microflora. This places a new idea that for the biosynthesis of nicotinic acid by the intestinal microflora even the inorganic nitrogenous compound in the form of ammonium ion can serve as the precursor.

Precursors of riboflavin.—Like the nicotinic acid the excretion of riboflavin is not increased by feeding excess of casein ; rather the excretion is decreased in this case showing (1) that the protein or the amino acid content of the food does not form the precursor for the biosynthesis of riboflavin and (2) that riboflavin is required for the utilisation of protein and its demand is rather increased with the increase of the protein level (26, 27, 69, 13).

In the laboratory, riboflavin is synthesized from alloxan and barbituric acid and these were, therefore, fed to the rats to see whether the animals can perform the laboratory process in the body (25). These two compounds on the other hand are synthesized from the simple nitrogenous compound urea and so this compound was also fed to see if it can produce any effect. It has been found that out of these three compounds only urea can stimulate the process of microbial synthesis of riboflavin in the intestine but the intermediates for such synthesis is not yet known. It is further revealed from these observations that alloxan and barbituric acid do not constitute the intermediates in the synthesis of riboflavin in the body. Although a great deal of work has been done regarding the precursors of nicotinic acid, only a few investigations have been made regarding riboflavin synthesis and researches in this line will yield fruitful results.

Precursors of other vitamins.--In the case of other vitamins as thiamine, pantothenic acid, etc., precursors for the synthesis have been studied mainly in the culture medium of the bacteria. In the case of thiamine it has been found that certain strains of bacteria require only the thiazole part, whereas some require the pyrimidine moiety of the thiamine molecule. Similarly in case of pantothenic acid it has been observed that certain strains can synthesize it if the amino acid alanine is given in the medium. Large-scale synthesis of this vitamin has been noted in the rumen of cows, sheep, etc.

Endogenous excretion of vitamins.—Regarding the source of the above precursors for the intestinal biosynthesis of the different vitamins by the microflora, it was previously conceived that some components of the diet as amino acids, etc., constitute the precursors for such microbial synthesis and the excretion of the different vitamins on a normal diet was regarded as exogenous in origin—partly being the metabolic fraction of the dietary supply and partly, especially the faecal output being the bacterial product synthesized from the precursors available from the dietary supply. De and Banerjee (17) however first conceived in their investigations on the mutual influence of riboflavin and nicotinic acid in metabolism that the bacterial synthesis may also be endogenous in origin and in the later investigations De and Datta (19) and De and Roy (20) have conclusively substantiated the previous concept of the endogenous origin of vitamins. It has been shown by feeding the adult rats that they excrete a large amount of nicotinic acid and riboflavin even when they are kept on diets free from these growth factors for a long period and the excretion under such conditions was regarded as endogenous in origin. It has been shown that the protein moiety of the different digestive enzymes form the precursors for the synthesis of these vitamins by the microorganisms when these are kept starved on dietary precursors of amino acids or other food constituents. Further work in this line is necessary to get more precise information as to how much quantity of the protein moiety of the different digestive enzymes are broken down to build the body protein of the intestinal microflora and for synthesis of the different vitamins by them.

BIOSYNTHESIS OF VITAMINS IN THE TISSUES

The fact that the vitamins are synthesized not only by the microflora in the intestine but also in other tissues has been realised only recently after the observations by Schweigert *et al.* (74) that injection of tryptophane in the chick embryo increased its nicotinic acid content and of Ling *et al.* (48) that the level of pyridine nucleotide in erythrocytes increased after injection of tryptophane in rats. De and Datta (19) in their investigations on the tryptophane-niacin relationship in rats has also suggested the possibility of partial conversion tryptophane to nicotinic acid in some tissues as well. This concept has been fully substantiated by the further work in this line by De and Guha (21, 22) with *in vitro* tissue culture experiment with liver and kidney tissue slices incubated with tryptophane in which it has been observed that these two tissues possess the capacity to convert tryptophane to nicotinic acid under aerobic conditions—the effect being higher in this case of liver than in kidney. This gives a direct proof of the tissue synthesis ef vitamin. Further work in this line should be undertaken to study if the different tissues can synthesize the vitamins specially of the B-group and how.

Tissue synthesis in relation to malignancy.—Tumour formation or carcinogenesis is influenced not only by the carcinogenic compounds or the viruses but also by the dietary factors such as vitamins, fat, etc. It has been observed that in certain cases of tumour when one vitamin accelerates the malignancy the same also possesses retarding effect in case of the others. So, as a rule, the vitamins act both carcinogenically and anticarcinogenically, depending on the nature of the tumour, the carcinogen and the species of the animal. As an example, dietary riboflavin exerts beneficial effect when given to rats suffering from hepatoma induced by p-dimethylaminoazobenzene but the same vitamin acts as an aggravating agent in the case of spontaneous mammary tumour in adult mice. There are several other instances and the whole literature in this field has been reviewed elsewhere (90). These pro and anticarcinogenic effects of a particular vitamin in relation to the different type of the tumors suggest that vitamin requirements for the patients suffering from cancer is much more divergent from the normal ones, depending on the nature of the tumour, in some cases the requirements are higher and in other cases lower.

Analysis of a large number of tumour tissues have revealed that their vitamin contents, different enzymatic activities, amino acid make-up of the proteins and their metabolic behaviour *i. e.*, respiration, glycolytic and other capacities are quite different from the tissues of the origin and this suggests that tumours represent a distinct tissue entity having the vitamin content, chemical make-up and the enzymatic activities different from the parent ones. In the process of carcinogenesis the carcinogenic compounds seem to produce changes in the metabolic activities required in the normal mitotic processes with the ultimate formation of a mutant just in the same way as in case of microorganisms and so the enzymatic activities and the vitamin contents are greatly influenced and the new requirements for these constituents are therefore set up in the mutant tumour tissue.

Every tissue possesses capacities for the synthesis and breakdown of a number of essential constituents and if the tumour actually represents distinct tissue entity, it is expected that their capacities for the synthesis and degradation will also undergo permanent changes under the influence of the carcinogens. With this possibility in view some experiments have been started to see how far the changes of the above capacities are brought about in the tumour cells.

Investigations by De and Guha (21, 22) show that the liver and kidney tissues possess capacities to synthesize nicotinic acid and this prompted the author and his collaborator (De and Guha, 20) to study how far the different carcinogens causing hepatoma can bring about changes in the above synthetic capacity and it has been found that p-dimethylaminoazobenzene, O-aminoazotoluene, benzpyrene, and 1, 2 : 5 : 6-dibenzanthracene inhibit the above process for the nicotinic acid synthesis—a necessary constituent for many dehydrogenase systems in the ordinary tissue cells.

More work has been started in this line and this will lead us to understand the mechanism by which the carcinogens actually elaborate their carcinogenic activities. When the data in this line will be sufficiently accumulated it will be possible to explore the compounds which will specifically act as anticarcinogens and thus a new line will possibly be devised for the treatment of cancers.

Moreover, the comparisons of the vitamin and enzyme patterns of the tumour tissue with those of the parent ones will help us to evaluate the significance of various enzyme systems to growth and to specialisation and to understand how one or more of these systems are affected by the carcinogens. Thus in future the study of the enzymatic pattern of the malignant tissue will help easily and in greater detail the diagnosis, prophylaxis and therapy of cancer and so our field of survey will be concentrated in smaller area than at present.

REFERENCES

1. Axelrod, A. E., Gross. P., Bosse, M. D. and Swingle, K. F. (1943). *J. Biol. Chem.* **148** : 721.
2. Beadle, G. W., Mitchell, H. K. and Nyc, J. F. (1947). *Proc. natl. Acad. Sci* , **33** : 155.
3. Benesch, R. (1945). *Lancet*, **1** : 718.
4. Black, S., Mc. Kibbin, J. M., and Elvehjem, C. A. (1941). *Proc. Soc. Exptl. Biol. Med.*, **47** : 308.
5. Black, S., Overman, R. S., Elvehjem, C. A. and Link, K. P. (1942). *J. Biol. Chem.*, **145** : 137.
6. Bonner, D. M. and Beadle, G. W. (1946). *Arch. Biochem.*, **11** : 319.
7. Bonner, D. M., and Yanofsky, C. (1949) *Proc. natl. Acad. Sci.* **35** : 537.
8. Bovarnick, M. R. (1943). *J. Biol. Chem.*, **151** : 467.
9. Bovarnink, M. R. (1944). *Ibid.*, **153** : 1.
10. Burkholder, P. R. and Mcveigh, I (1942). *Proc. natl. Acad Sci.*, **28** : 285.
11. Cartwright, G. E., Wintrobe, N. M., Jones, P., Lauristen, M. and Humpreys, S. (1944). *Bull. John Hopkins. Hosp.*, **75** : 35.
12. Cohendy, M. (1912). *Ann. Inst. Pasterus.*, **26** : 106.
13. Czaczkes, J. W. and Guggenheim, K. (1946). *J. Biol. Chem.*, **162** : 267.
14. Daft, F. S. Ashburn, L. L. and Sebrell, W. H. (1942). *Science*, **96** : 321.

15. Daft. F. S. and Sebrell, W. H. (1943). *U. S. Pub. Health. Repts.,* **58** : 1542.
16. Daft. F. S. and Sebrell, W. H. (1945). Vitamins and Hormones, **3** : 49.
17. De, H. N. and Banerjee, S. N. (1949). *Indian J. Med. Res.,* **37** : 293.
18. De, H. N. and Datta. P. K. Jr. (1950). *Curr. Sci.,* **19** : 279.
19. *Ibid.* (1951). *Indian J. Med. Res.,* **39** : 63.
20. De, H. N. Datta, P. K. and Roy. J. K. (1951). *Ibid.,* **39** : 353.
21. De, H. N., and Guha, S. R. (1951). *Ibid.,* **39** : 337.
22. *Ibid.* (1951). *Ibid.,* **39** : 343
23. *Ibid.* (1950). *Brit. J. Cancer,* **4** : 430.
24. De, H. N., Malaker, M. C. and Paul, A. K. (1949). *Indian. Med. Gaz.,* **84** : 542.
25. De, H, N. and Roy. J. K. (1950). *Curr. Sci.,* **19** : 241.
26. *Ibid.* (1951). *Indian Med. Res.,* **39** : 73.
27. *Ibid.* (1951). *Ibid,,* **39** : 83.
28. Ellinger, P. Benesch, R. and Kay, W. W. (1945). *Lancet,* **1** : 432.
29. Ellinger, P. Coulson, R. A. and Benesch, R. (1944). *Nature,* **154** : 270.
30. Ellinger, P and Coulson, R. A. (1944). *Biochem. J.,* **38** : 265.
31. Ellinger, P. and Kader, A. M. M. (1949). *Biochem. J.,* **44** : 285.
32. Feldt, A. (1941). *Klin. Wochschr.,* **20** : 945.
33. Fildes, P. (1940). *Lancet,* **1** : 955.
34. Green, D. E., Leloir, L. F. and Nocito, V. (1945). *J. Biol. Chem.,* **161** : 559.
35. Guerrant, N. B. and Dutcher, R. A. (1932). *Ibid.,* **98** : 22.
36. *Ibid.* (1934). *Proc. Soc. Exptl. Biol. Med.,* **31** : 796.
37. Guggenheim, M. (1940). *Die Biogenin. Amine. 2nd. Ed.* 174.
38. Hawking, F. (1944). *Brit. J. Exptl. Path.,* **25** : 63.
39. Heidelberger, C., Gulberg, M. E., Mergan, A. F. and Lepkovsky, S. (1948) *J. Biol. Chem.,* **175** : 417.
40. *Ibid.* (1949). *Ibid.,* **170** : 143, 151.
41. Huff, J. W. and Perlzweig, W. A. (1942), *Ibid.,* **142** : 401.
42. Juenqueria, P. S. and Schweigert, B. S. (1948). *Ibid,,* **175** : 535.
43. Kornberg, A., Datt, F. S. and Sebrell, W. H. (1943), *Science,* **98** : 20.
44. *Ibid.* (1944). *U. S. Pub. Health Reports.* **59** : 832.
45. Krehl, W. A., Hendersen, L. M., Huerga, J., De, La and Elvehjem, C. A. (1946). *J. Biol. Chem.,* **166** : 531.
46. Krehl. W. A., Sarma, P. S. and Elvehjem, C. A. (1946a). *Ibid.,* **162** : 403.
47. Krehl, W. A. Sarma, P. S., Tepley L. J. and Elvehjem, C. A. (1948) *J. Nutr.,* **31** : 85
48. Ling, C. T., Hegsted, D. M. and Stare, E. C. (1948), *J. Biol. Chem.,* **174** : 803.
49. Lichstein, H. C., Gunsalus, I. C. and Umbreit, W.W. (1945). *J. Biol. Chem.,* **161** : 311.
50. Mannering, C. J.. Orsini, D. and Elvehjem, C. A. (1944), *J. Nutr.,* **28** : 141.
51. Marshall, E. K. Jr., Bratton, A. C.; White, H. J. and Litchfield J. T. (1940). *Bull. John. Hopkins. Hosp.* **67** : 163.
52. Milhorat. A. T. and Bartels, W. E. (1945). *Science,* **101** : 93.
53. Mitchell, H. K. and Isbell, E. R. (1942), *Univer. Texas. Publication,* **No. 4237**, 125.
54. Mitchell, H. K., Nyc. J. F. and Owen, R. D. (1948). *J. Biol. Chem.,* **175** : 433.
55. Najjar, V. A. and Barrett, R. (1945), Vitamins and Hormones. **3** : 23.
56. Najjar, V. A. and Holt, L. E. (1943). *J. Amer. Med. Assoc.,* **123** : 683.,
57. Najjar, V. A., Johns, G. A., Mediary G. C., Fleischman, O. and Holt, L. F. (1944) *Ibid.,* **126** : 357.
58. Nielsen, E. and Elvehjem. C. A. (1942). *J. Biol. Chem.,* **145** : 713.
59. Nielsen, E. and Black. A. (1944). *Proc. Soc. Exptl. Pathol.,* **29** : 455.
60. Nimmo-Smith, R. H., Lascalles. J. and Woods, D. D. (1948). *Brit. J. Exptl. Pathol.,* **55** : 14.
61. Nuttall, G. H., Thiefelder, H. (1895-96). *Physiol. Chem.,* **21** : 109.

62. O'Kane, D and Gunsalus, I. C. (1947), *J. Biol. Chem.*, **170:** 425.
63. Osborne and Mendel (1911). *J. Clin. Invest.*, **26** : 112.
64. Pacini and Russel. (1935). *J. Bact.*, **24** : 118.
65. Perlzweig, W. A., Rosen, F., Levitas, N. and Robinson, J. (1947). *J. Biol. Chem.* **167** : 511.
66. Rosen, L., Huff, J. W. and Perlzweig, W. A. (1946). *J. Biol. Chem.*, **163 :** 343.
67. Rossen, E. Huff, J. W. and Perlzweig, W. A. (1947). *J. Nutr.*, **33** : 561.
68. Sarett, H. P. and Goldsmith, G. A. (1947). *J. Biol. Chem.*, **167** : 293.
69. Sarett, H. P. and Perlzweig, W. A. (1943). *J. Nutr.*, **25** : 173.
70. Schlenk, F. and Fisher, A. (1947). *Arch. Biochem.*, **12 :** 69.
71. Schlenk, F. and Snell, E. E. (1945). *J. Biot. Chem.*, **157** : 425.
72. Schweigert, B. S. and Mc Intire, J. M. (1945). *Arch. Biochem.*, **6** : 403.
73. Schweigert, B. S and Pearson, P. B. (1947). *J. Biol. Chem.*, **168** : 555.
74. Schweigert, B. S. and Pearson, P. S. (1948). *Ibid.*, **172** : 485.
75. Schweigert, B. S., Pearson, P. B. and Wilkening, M. C. (1947). *Arch. Biochem.*, **12** : 139.
76. Schweigert. B. S. Tepley, L. J., Greenhut, I. and Elvehjem, C. A. (1945). *Amer. J. Physiol.* **144** : 74.
77. Skeggs, H. R. and Wright, L. D. (1946). *J. Nutr.*, **32** : 375.
78. Snell, E. E. (1944). *J. Biol. Chem.*, **154 :** 313.
79. Snell, E. E. (1945). *J. Amer. Soc.*, **67 :** 194.
80. Snell, E. E. and Rannefeld, A. N. (1945). *J. Biol. Chem.*, **157 :** 475.
81. Spies, S. S. Daft, F. S., Sebrell, W. H. and Ashburn, L. L. (1942). *U. S. Pub. Health Repts.*, **57 :** 1559.
82. Tamura, J. T. (1944). *J. Bact.* **47** : 529.
83. Thompson, R. C. (1942). *Univer. Texas Publ.* **No. 4237,** 87.
84. Theiler, A., Green, H. H. and Viljoen, P. R. (1915). *Rep. Vet. Res. South Africa.*, **Nos. 3, 4 & 9.**
85. Umbreit, W. W., O'Kane, D. J. and Gunsalus, I. C. (1946). *J. Bact.*, **51** : 576.
86. Welch, A. D. (1942). *Fed. Proc. Fed. Proc.* **1** : 173.
87. West, H. D.: Jefferson, N. C. and Riveria, R. E. (1943). *J. Nutr.*, **25** : 471.
88. Wright, L. D. and Welch, A. D. (1943). *Science*, **97** : 426.
89. Welch. A. D. (1948), *Physiol. Rev.*
90. De. H. N. (1950.) *Med. Digest, 18,* (*No* 9), 427.

THE EFFECT OF NITROGEN FERTILIZATION ON THE CHEMICAL COMPOSITION OF THE LEAVES OF PEACH TREES

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The value of nitrogenous fertilizers for most fruit crops has long been recognized and at no time has there been a greater need for a sound understanding on profitable use of fertilizers than at the present day. The amounts of various nutrient elements present in the assimilating leaves should be adequate for their normal functioning. The chemical analysis of the leaves also reflects the behaviour of each ingredient on the utilization and accumulation of others. Cullinan, Scott and Waugh[4] reported that in all series where N was high, the nitrogen content of the peach leaves ran uniformly high and was lower as the N content of the nutrient solution decreased. The reduction in the nitrogen content also appears to be associated with the age of the leaves. Nasharty[8] observed a seasonal decline in the nitrogen content of the leaves of peach trees.

The probable effects of nitrogen on the content of other nutrient elements have been studied by various investigators. Lilleland and Brown[7] reported that the phosphorus content of the peach leaves varied markedly with nitrogen fertilization, exhibiting a reciprocal relationship. Similarly, Cullinan and Batjer[5] observed an increase in P as the nitrogen decreased in the foliage of peach trees. Potassium content of peach leaves of all trees in nitrogen series was uniformly high and appeared independent of N content (Cullinan, Scott and Waugh[4]). Brown[3] and Cullinan and Batjer[5], however, reported that potassium decreased with an increase in nitrogen supply.

The effects of N on the calcium and magnesium content of the peach leaves have not been so thoroughly investigated. Cullinan and Batjer[5] reported that a decrease in Ca occurred with increasing concentration of nitrogen. Brown[3] suggested that larger accumulation of Mg resulted where nitrogen increased in the shoots of the peach trees.

The study of the composition of the leaves and the changes in their composition during the growing season would provide direct information on the nutritional status of the plants. It would also prove to be of great advantage in planning fertilizer programmes to meet the future requirements of the trees without any visible evidence of deficiency symptoms. Therefore, the present investigation was carried out to study the effects of five levels of nitrogen applied to peach trees, growing in sod under orchard conditions, on the chemical composition of the foliage with respect to nitrogen, phosphorus, potassium, calcium and magnesium content at three different stages during the growing season.

MATERIALS AND METHODS

The peach trees used in this investigation were planted in the year 1945 and trained to modified leader system. Light to moderate pruning has been practised throughout the life of the plants. Inorganic commercial fertilizer, ammonium sulphate, with an average chemical composition 20·5 per cent N was used. An application of 0·05 lb. of N for each year of the age of the tree is regarded as normal amount and will be referred to as the 1 N treatment. Five different treatments, ½ N, 1 N, 2 N, 4 N and 2 N+2 N, the last being the split application, were used. The required amount of the fertilizer was spread under the trees in a uniform broadcast band, starting one foot from the trunk and extending outward to about one foot beyond the drip of the branches. The fertilizer application was made on April 19, 1948, except for the split application treatment, where 0·10 lb. of N was applied on April 19, and the remaining 0·10 lb. of N on June 28, 1948. No other fertilizer treatments have been made in this orchard. The treatments have been replicated thrice with the exception of ½ N treatment, which had only two replications. Four trees are included under each replicate.

The orchard soil is silt loam and is relatively uniform in depth with the "A" horizon extending to 6—8 inches deep and the "B" horizon to approximately 24—30 inches, below which is parent material. Its productivity rating for field crops is 3·5 on the basis of a scale from 1 to 10, with one indicating the most productive and 10 the least productive soil. For orchard purposes it is rated excellent.

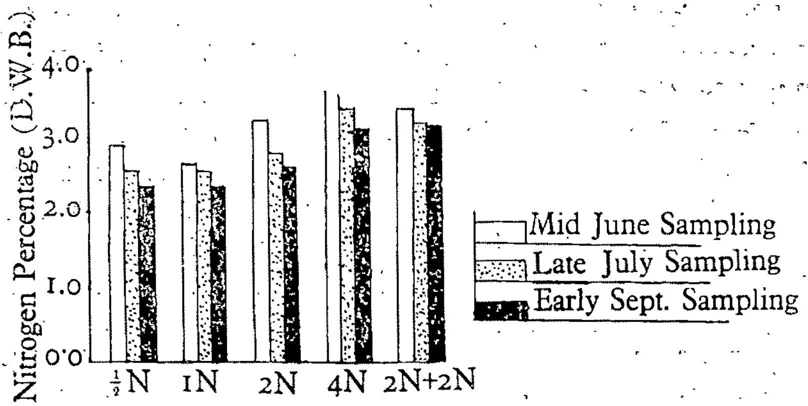

FIG. 1. Showing the influence of N-fertilizer on the percentage of total nitrogen.

In the present work seventy leaves were picked at random by walking around the trees and removing one or two leaves from the middle of the current season shoots. Sampling was done at three different dates, namely, during mid June, late July and early September 1948.

Table 1. Showing the influence of N-fertilizer on the percentage of total nitrogen.

Treatment	Mid June	Late July	Early Sept.	Mean	Increase or decrease
½ N	2·94	2·59	2·39	2·64	
1 N	2·72	2·62	2·40	2·57	–0·07
2 N	3·30	2·86	2·66	2·94	0·37
4 N	3·72	3·44	3·17	3·44	0·50
2 N+2 N	3·44	3·14	3·22	3·30	–0·14
Mean	3·23	2·93	2·77	2·98	
Increases		–0·30	–0·16		

*Per cent on dry weight basis.

L. S. D.=0·083

The total amounts of N, P, K, Ca, and Mg were determined by the methods used by the Association of Official Agricultural Chemists[1], with the exception that potassium was analysed by sodium cobaltinitrite method[2]. Hydrogen peroxide was used for ashing the leaf powder obtained by grinding the dried leaf samples.

Experimental Findings

EFFECT OF NITROGEN FERTILIZER ON NITROGEN CONTENT

The direct influence of N fertilizer on the percentage of total nitrogen calculated on the dry weight basis is very well exhibited by the observations recorded in table 1 and represented in quadrangular blocks in Fig. 1. Leaves from the plants receiving 4 N treatments contained the highest amount of nitrogen and the total leaf N decreased as the amount of N fertilizer was reduced. The reduction in the mean value of nitrogen was as much as 0·8 per cent under ½ N when compared to 4 N treatment. There was a gradual decline in N percentage with the advance of season. Leaf nitrogen under the split application (2 N+2 N) fluctuated least and was maintained at a fairly high level throughout the growing season.

PHOSPHORUS CONTENT

The data portrayed in table 2 and diagrammatically shown in Fig. 2 indicate that an increase in N fertilization was closely associated with a slight reduction in the P_2O_5 content of the mid shoot leaves. Unlike the nitrogen content, the percentage of P_2O_5 increased a bit in the leaves at the time of third sampling. No symptoms of phosphorus deficiency appeared during the growing season.

Table 2. P_2O_5 content* of leaves of peach trees receiving different amounts of Nitrogen fertilizer.

Treatment	Mid June	Late July	Early Sept.	Mean	Increase or decrease
½ N	0·74	0·65	0·71	0·70	
1 N	0·73	0·62	0·90	0·75	0·05
2 N	0·71	0·55	0·63	0·63	−0·12
4 N	0·66	0·55	0·62	0·61	−0·02
2 N+2 N	0·71	0·54	0·60	0·62	0·01
Mean	0·71	0·58	0·69	0·66	
Increases		−0·133	0·11		

*Per cent on dry weight basis

L. S. D. = ·0098.

POTASSIUM CONTENT

On an average the potassium content of the leaves was fairly high. An inspection of table 3, would indicate that nitrogen had slightly depressing effect on K content of the mid shoot leaves. There were only minor fluctuations in the potassium percentage during the growing season and the leaves were reasonably rich in this nutrient.

CALCIUM CONTENT

The data in table 4, show the calcium content of the mid shoot leaves. Calcium percentage was more or less alike in treatments ½ N and 1 N, but with 2 N it increased by about 6·5 per cent over the 1 N treatment. Any further increase in N seems to have a depressing effect on the Ca content of the leaves. Calcium in the leaves increased with the advance in the growing season and this was relatively higher under lower levels of nitrogen. Fig. 3 shows the trend in Ca content as observed during the growing season.

Table 3. Potassium content* of leaves of peach trees receiving different amounts of Nitrogen fertilizer.

Treatment	Mid June	Late July	Early Sept.	Mean	Increase or decrease
½ N	4·29	4·74	4·11	4·38	
1 N	4·14	4·51	4·29	4·31	–0·07
2 N	4·01	4·34	4·12	4·16	–0·15
4 N	3·87	4·28	4·15	4·10	–0·06
2 N+2N	4·09	4·19	4·08	4·12	0·02
Mean	4·08	4·41	4·15	4·22	
Increases		0·33	–0·26		

*Per cent on dry weight basis

L. S. D. = ·078

MAGNESIUM CONTENT

The observations recorded in table 5, clearly show an increase in the Mg content of the mid shoot leaves by about 25 per cent under 4 N when compared to ½ N treatment at the first sampling. In the subsequent samplings the differences were not so conspicuous. Mg content of the leaves declined with an advance in the growing season and at a rapid rate from July onwards.

The data of total N, P_2O_5, K, Ca, and Mg content of leaves of peach trees receiving different amounts of nitrogen fertilizer, when subjected to statistical analysis, revealed significant differences due to treatments and time of sampling at one per cent level.

Table 4. Calcium content* of leaves of peach trees receiving different amounts of Nitrogen fertilizer.

Treatment	Mid June	Late July	Early Sept.	Mean	Increase or decrease
$\frac{1}{2}$ N	1·59	2·30	2·69	2·19	
1 N	1·58	2·35	2·60	2·18	–0·01
2 N	1·66	2·46	2·85	2·32	0·14
4 N	1·32	1·82	2·11	1·75	–0·57
2 N+2N	1.65	2·34	2·36	2·11	0·36
Mean	1·56	2·25	2·52	2·11	
Increases		0·69	0·27		

*Per cent on dry weight basis

L. S. D.=·070.

DISCUSSION

The nitrogen content of the mid shoot leaves varied according to the different levels of N application. The minimum nitrogen content recorded under $\frac{1}{2}$ N treatment was as low as 2·39 at the third sampling. The appearance of nitrogen deficiency symptoms on these trees late in the season leads one to conclude that N percentage from 2·0 to 2·5 accompanied by fairly large quantities of other nutrient elements in the mid shoot leaves of peach trees is the minimum level and any further reduction is likely to be reflected in characteristic visual nitrogen deficiency symptoms under the prevailing conditions of this experiment.

It was surprising that the mean values for total nitrogen content under treatment 1 N should decline instead of showing an increase in comparison to $\frac{1}{2}$ N treatment. The possible explanation seems to be that the increased nitrogen fertilization stimulated vegetative growth and initiated better fruit set in treatment 1 N, thus preventing higher concentrations in the leaves. If, however, the values recorded for July sampling are examined a regular increase in nitrogen percentage is observed with all the treatments.

Table 5. Magnesium content* of leaves of peach trees receiving different amounts of Nitrogen fertilizer.

Treatment	Mid June	Late July	Early Sept.	Mean	Increase or decrease
½ N	0·74	0·72	0·48	0·64	
1 N	0·86	0·78	0·51	0·72	0·08
2 N	0·92	0·82	0·45	0·73	0·01
4 N	0·93	0·81	0·43	0·72	–0·01
2 N+2 N	0·87	0·73	0·42	0·67	–0·05
Mean	0·86	0·73	0·46	0·70	
Increases		–0·13	–0·27		

*Per cent on dry weight basis

L. S. D.=·0186

In the leaves sampled after harvest, *i.e.*, early September, the nitrogen content under split application (2 N+2 N) was 28 per cent higher than the plants having the least amount of nitrogen, ½ N treatment. There is every possibility that this extra amount of nitrogen in such plants can greatly help in proper development of the tissues, which being well matured should be capable of withstanding cold without severe winter injuries.

Although the value of P_2O_5 slightly decreased in the mid shoot leaves with increases in nitrogen, the absolute amount of phosphorous present in the foliage does not seem to deviate much among the different treatments if the increase in dry weight of the tissues owing to excessive vegetative growth is given due consideration. It has been reported that the use of ammonium sulphate as nitrogen fertilizer actually accelerates the absorption of P_2O_5 (Dikussar[6]). Plants receiving higher amounts of N apparently absorbed larger total amounts of phosphorus than those receiving less nitrogen.

The phosphorus content which varied from 0·54 to 0·65 per cent under different treatments, seems to be enough for normal fruit production in this orchard. No phosphorus deficiency symptoms appeared on the leaves. Changing amounts of P_2O_5 hardly seem to influence the percentage of any other nutrient element as far as their amount in mid shoot leaves is concerned.

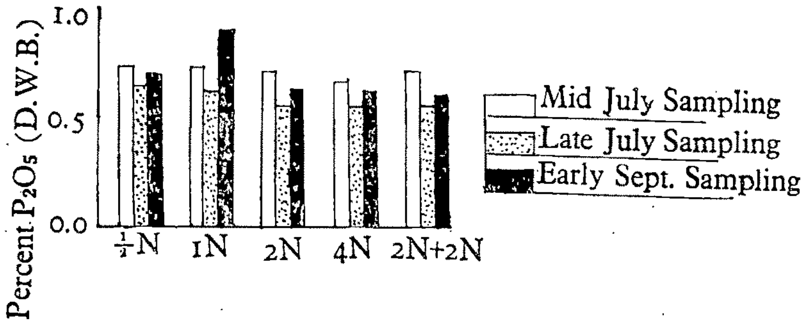

FIG. 2. P_2O_5 content influenced by N-fertilizer.

There was a consistent reduction in the percentage of potassium with increasing amounts of N in the leaves, the lower values still remain far above the critical range. Luxuriant vegetative growth with heavier nitrogen applications may partly be accounted for reduction in K percentage. There is no doubt that concentration of K per unit area decreased as the amount of nitrogen fertilizer was increased. This decrease in K appears to be dilution effect. Increasing amounts of K appear to have a depressing effect on the magnesium content of the leaves.

The relative amounts of different nutrient elements as revealed by the chemical analysis of the leaves computed as mean values have been graphically shown in Fig. 4.

Increase in the nitrogen level from ½ N to 2 N encouraged the accumulation of Ca in the mid shoot leaves. Any further increase in N had a depressing effect on the calcium content of the leaves. The relative amounts of Ca to N and K also increased with the advance in the growing season. Leaves containing 1·30 per cent calcium appeared to be as healthy as those having higher amounts of Ca.

The quantitative analyses of the leaf tissue do not clearly indicate whether 1·30 per cent calcium was a result of deficiency of Ca available in the soil or the effect of high concentrations of K in the leaves or due to reasons of soil acidity. A lack of boron in the medium has been shown to reduce the uptake of calcium (Warington[9]).

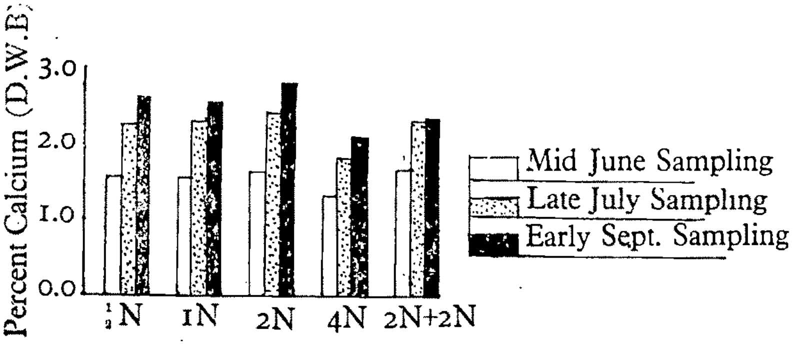

FIG. 3. Influence of N-fertilizer on the Calcium-content.

Magnesium content in the mid shoot leaves was fairly high during the mid June and late July sampling. It tended to increase in the leaves, at least to some extent, with an increase in nitrogen application. It seems that owing to its high mobility it readily migrates from the older leaves to other plant parts. In spite of a high concentration of K, the Mg content throughout the sampling period was above the deficiency level. Lower Ca content and increasing N fertilization apparently accelerated the accumulation of Mg in the mid shoot leaves. Decrease in nitrogen and increase in Ca with the advance of season are also associated with a marked reduction in magnesium content of the leaves.

SUMMARY AND CONCLUSIONS

During the past few decades various investigators have emphasized the importance of the plant analysis for a better understanding of their nutritional status. The present investigation was conducted to study the effects of nitrogen applied to peach trees growing in sod under orchard conditions on the chemical composition of the leaves with respect to total N, P_2O_5, K, Ca, and Mg content at three different stages during the growing season.

Total nitrogen content of the mid shoot leaves increased with increase in N fertilization and varied from 2·39 to 3·72 per cent. Nitrogen deficiency symptoms appeared on plants receiving $\frac{1}{2}$ N treatment by the end of July. N percentage from 3·0 to 2·5 in young peach trees appears to be the minimum level and any further reduction is likely to be reflected in characteristic visual deficiency symptoms under the prevailing condition of the experiment. 2 N treatment appears to be satisfactory.

There was a gradual decline in the nitrogen content with advance of the season. On an average the reduction in N content of the mid shoot leaves from mid June to early September amounted to 0·46 per cent.

The P_2O_5 content slightly decreased with an increase in N and ranged from 0·54 to 0·90 per cent. It seemed enough for normal fruit production and no symptoms of phosphorus deficiency appeared on the leaves during the growing season. Variations in the amount of P_2O_5 had no influence on the percentage of other nutritive elements.

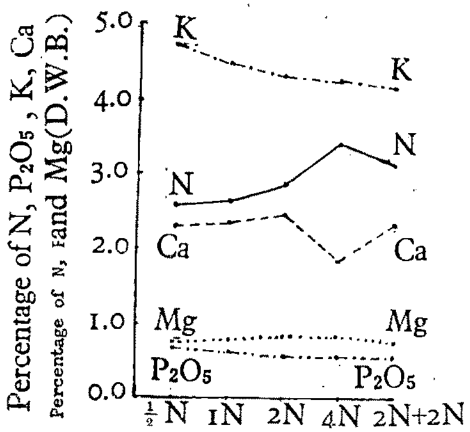

FIG. 4. Influence of N-fertilizer on chemical composition of peach leaves.

There was a consistent reduction in percentage of K with increasing amounts of N and luxuriant vegetative growth induced by heavier N fertilization may be partly accounted for this reduction. In other words the decrease in K appears to be more or less a dilution effect. The average potassium content ranged from 3·87 to 4·74 per cent on the dry weight basis.

Increase in N from $\frac{1}{2}$ N to 2 N encourged accumulation of calcium in the mid shoot leaves. Leaves with 1·30 per cent Ca appeared to be healthy. The relative amounts of Ca to N and K also increased with advance in the growing season.

Magnesium content tended to increase with additional nitrogen applications. In the third sampling made after the harvest of fruits the Mg percentage declined and ranged between 0·42 to 0·51 per cent. Lower calcium and increasing N fertilization seem to have accelerated the Mg accumulation in the mid shoot leaves. Increasing amounts of K appear to have a depressing effect on the magnesium content of the leaves.

From the results of this investigation application of nitrogen at the rate of 0·1 lb. of N for each year of age of the tree appeared desirable for young peach trees growing in sod under orchard conditions.

ACKNOWLEDGMENTS

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REFERENCES

1. Association of Official Agricultural Chemists. Methods of Analysis, Sixth Edition (1945), A.O.A.C., Washington, D.C., U.S.A.
2. Brown, D. C., R. R. Robinson and G. M. Browning. 1938. Determination of Small Amounts of Potassium. *Ind. & Eng. Chem. Ana. Edi.*, **10** : 652-654.
3. Brown, D.S. 1945. The Growth and Composition of the Tops of Peach. Trees in sand culture in Relation to Nutrient Element Balance. *West Virginia Agric. Expt. Stat. Bull.*, **322**.
4. Cullinan, F.P., D.H. Scott and J.G. Waugh. 1938. The effects of Varying Amounts of Nitrogen, Potassium and Phosphorus on the Growth of Young Peach Trees. *Proc. Amer. Soc. Hort. Sc.*, **36** : 61-68.
5. Cullinan, F.P. and L.P. Batjer. 1943. Nitrogen, Phosphorus and Potassium Inter-relationships in Young Peach and Apple Trees. *Soil Sci.*, 55.
6. Dikussar, I.G. 1937. The Physiological significance of Ammonium Salt in relation to the Composition Changes of the Nutrient Solution. *Soil Sci.*, **50** : 67-76.
7. Lilliland, O. and J.G. Brown 1942. The Phosphate Nutrition of Fruit Trees. *Proc. Amer. Soc. Hort. Sci.*, **41** : 1-10.
8. Nasharty, A.H. 1944. Accumulation of Nitrogen from Different Sources by Peach Trees. *Proc. Amer. Soc. Hort. Sci.*, **45** : 5-8.
9. Warington, K. 1934. Studies in Absorption of Calcium from Nutrient Solutions with special reference to the presence or absence of boron. *Ann. Bot.*, **48** : 743-775.

PREDICTIONS OF THE IONOSPHERIC CONDITIONS FROM THE OBSERVATIONS OF FADING PATTERN OF RADIO-SIGNALS

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The study of the fading patterns of short wave radio-signals from Indian as well as foreign stations was carried out by the author in the Communication Laboratory of the Engineering College, Benares Hindu University for a number of years. The fading patterns are classified according to their mode and theory of formation. The present communication is intended to show the relation between the fading patterns and the ionospheric conditions, particularly with respect to the electronic concentration existing there at the time of observation.

The accumulated data of the observation of fading patterns (both visual and automatic-ink record) are classified into the following groups:—

1. Peaky random.
2. Non-peaky or slow random.
3. Quasi-periodic.
4. Slow and smooth periodic (magneto-ionic fading)
5. Peaky periodic.
6. Slow periodic, superimposed with ripples
7. Complex periodic.

Typical fading patterns characteristic of the types mentioned above are shown in figs. 1—6. The following table shows the agreement of the electronic density in the F2-region with the occurrence of such patterns at Benares on 19 metre band transmission from A.I.R., Delhi.

Fig. No.	Date & Time in I.S.T.	Nature of fading.	Existing elec/cc in F2-region.	Electron/cc required in single reflection F 2.	Elec/cc required for double reflection from F2-region
1	18.5.46 1054 Hrs.	Peaky random	$1{\cdot}44\times10^6$	1.42×10^6 to 1.57×10^6	Between $1{\cdot}99\times10^6$ and 2.25×10^6
2	6.4.47 1718 Hrs.	Slow random & quasi-periodic.	$2{\cdot}60\times10^6$	do.	do.
3	30.5.48 1750 Hrs.	Peaky periodic.	$2{\cdot}40\times10^6$	do.	do.
4	12.5.47 1859 Hrs.	Slow and smooth periodic magneto ionic.	$1{\cdot}04\times10^6$	do.	do
5	20.10.48 0735 Hrs.	Slow & smooth periodic superimposed with ripples.	$1{\cdot}8\times10^6$	do.	do
6	4.4.48 0745 Hrs.	Complex periodic.	$1{\cdot}49\times10^6$	do.	do

The electronic density (column 4) in the F2-region at the time of observation was calculated from the ionospheric data supplied by the Research Department, A. I. R.. The electronic concentrations (columns 5 and 6) refer to single and double reflections respectively from the F2 region of the ionosphere for the distance between Delhi (Lat. 28°, 35′ N, long. 77°, S′E) and Benares Lat. 25°, 16′ N., Long. 83° 2′E) situated at a distance of 678·4 km. These electronic densitites in the F2 region required for single and double reflections for various bands have been calculated with the help of ionospheric charts kindly supplied to us by the Superintendent, National Physical Laboratory, Teddington, England. These densitites may also be calculated from the well known relation

$$\cos^2 \alpha = 1 - \frac{4\pi N e^2}{m\, w^2}$$

where α =angle of penetration of the wave in the ionosphere
N=electronic density
e and m=charge and mass of electron respectively
w=pulsatance of the wave.

Nature of Fading and its Relation with the Ionosphere

1. Peaky Random.—The most commonly occurring fading

pattern of received radio signals are of random nature, as has been observed by various investigators (1, 2, 5, 6). The random nature of fading has been explained by Ratcliffe and Pawsey to be due to scattering of energy at the ionosphere, which is made up of various diffracting centres. Fig. 1 represents a peaky random pattern. It will be seen from the table that the formation of purely peaky random type of fading requires the electronic densities in the ionosphere to be enough for single reflection. The electronic density required for single reflection of 19 metre band from F2-region of the ionosphere between Delhi and Benares as given in column 5 is 1.42×10^6 electrons per c.c., while according to column 4, the existing concentration of electrons at the time of observation was 1.44×10^6. A number of peaky random pattern of fadings at various wave bands (13, 16, 19, 25, 31, 41, 49 and 60m) were analysed by drawing the Rayleigh's intensity distribution curves and in all the cases existing electronic densities at the hour of observation were compared with those required for single reflection. The accumulated data suggest and confirm the conclusion that the occurrence of the peaky random fading on a particular wave band corresponds to a certain range of electronic density in the F2-region of the ionosphere. For instance, the appearace of peaky random pattern shown in Fi . 1 on 19m band will suggest to the observer that the electronic density in the ionosphere is fairly high (more than $1{\cdot}42 \times 10^6$ elec. c.c.) for the reception of 19 metre band and better still for higher wave lengths. Similarly the occurrence of this type of fading on 25, 31, 41 and so on metre bands predicts the electronic densities in the F2-region of the ionosphere as given in table II.

S.N.	Wave band	Electronic densities in electrons/c.c.
1	13 metre.	Between $2{\cdot}89 \times 10^6$ and $3{\cdot}36 \times 10^6$
2	16 ,,	,, $1{\cdot}96 \times 10^6$ and $2{\cdot}21 \times 10^6$
3	19 ,,	,, $1{\cdot}42 \times 10^6$ and $1{\cdot}57 \times 10^6$
4	25 ,,	,, $0{\cdot}84 \times 10^6$ and $0{\cdot}91 \times 10^6$
5	31 ,,	, $0{\cdot}53 \times 10^6$ and $0{\cdot}58 \times 10^6$
6	41 ,,	,, $0{\cdot}32 \times 10^6$ and $0{\cdot}33 \times 10^6$
7	49 ,,	,, $0{\cdot}23 \times 10^6$ and $0{\cdot}24 \times 10^6$
8	60 ,,	,, $0{\cdot}15 \times 10^6$ and $0{\cdot}16 \times 10^6$

2. Slow Random and (3) Quasi Periodic.—When the randomness in the fading pattern is decreased and peaky nature is considerably reduced, it is described as non-peaky or slow random. It may be pointed out that the formation of such fading patterns is caused if two waves reflected from two independent diffracting centres in the ionosphere reach simultaneously a receiving centre, the pattern obtained at the receiver will be the resultant of two independent random fading patterns and the resultant fading pattern so obtained may not be purely of random nature. As a consequence, the fading pattern assumes the nature called slow random. It may be concluded that the electronic density in the ionosphere during the hour of formation of such patterns on a particular frequency must be adequate to permit double reflection either from the same layer or from two different layers so that more than one reflection may reach the receiver. Under the conditions of the ionosphere appropriate for the formation of slow random fading pattern, if the layer possesses slow vertical movement, as it often does, particularly at the

Fig. I

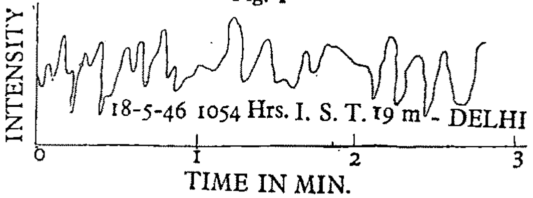


Fig. II

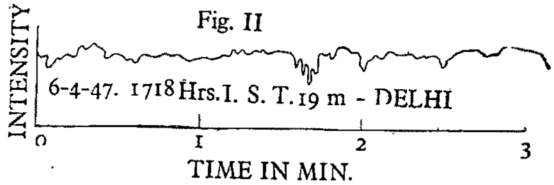
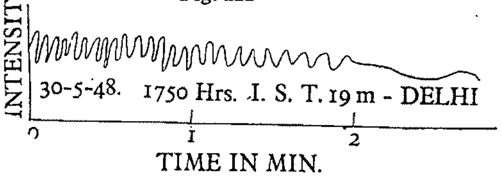


Fig. III



transition period of electronic density in the morning or evening hour, the resultant fading pattern will change from slow random to what has been described as quasi periodic. Fig. 2 represents a fading pattern of this type on 19 metre band. It will be seen from Table I that the electronic density in the ionosphere at the hour of observation is $2{\cdot}6 \times 10^6$ electrons/c.c. and density required for double reflection of the wave from F 2-region is between $1{\cdot}99 \times 10^6$ and $2{\cdot}25 \times 10^6$ electrons/c.c. Thus, the occurrence of this type of fading is associated with high electronic density and slow vertical movement of the layer. It may be pointed out that the occurrence of these types of fading patterns on a particular frequency predict that the ionosphere conditions are suitable for radio-communication on the frequency used.

PERIODIC FADING.—Periodic fading occurs distinctly under two different conditions (3, 7) of the ionosphere, namely, (1) when the electronic density in the ionospheric layer is fairly high to permit double reflections of the wave on a particular frequency and the layer or layers from where the reflections are taking place, possess vertical movement, (2) when the electronic density in the ionosphere is considerably low so as to be just sufficient for single reflection of the wave between the transmitting and receiving stations. The periodic fading under the first condition is caused by the interference between two waves either from the same or two different layers and is usually quick and peaky. The periodic fading under the second condition is formed due to the interference between the ordinary and extraordinary ray reflections of the split wave from the ionosphere due to magneto-ionic effect. They have therefore been called magneto-ionic fading. Such fading patterns are usually slow and smooth, hence they are also called slow and smooth periodic.

PEAKY PERIODIC.—Fig. 3 depicts a peaky periodic fading pattern on 19 metre band. The electronic density existing at the hour of observation was $2{\cdot}4 \times 10^6$ electrons/c.c. as shown in column 4 of table I. which is higher than the electronic density required for double reflection of 19 metre band as shown in column 6 of the same table. The rate of movement of F2-layer calculating from the fading pattern was 8·5 km. per hour (9).

The analysis of a large number of peaky periodic fading patterns on various short wave lengths (13, 16, 19, 25, 31, 41, 49 and 60 m) leads to the conclusion that the occurrence of this type of fading is undoubtedly accompanied by (1) high electronic density in the ionosphere to the extent of and above that needed for double reflection of the wave used and (2) vertical movement of the ionospheric layer or layers from which the reflections are occurring, which can be calculated from the fading pattern.

Thus, these types of fading patterns may go to predict the suitability of the ionospheric condition for radio communication on the one hand, and on the other, provide an easy method for calculating the rate of movement of the ionospheric layer.

4. SLOW AND SMOOTH PERIODIC (MAGNETO-IONIC) FADING.—Figure 4 represents an example of this type. It will be seen from table I that the electronic density in the F2-region of the ionosphere at the time of observation was just enough for single reflection of 19 metre band, which later decreased to $1{\cdot}04 \times 10^6$ electrons/c.c., a value which is less than what is needed for single reflection of the wave. Consequently there is a complete disappearance of the signal as shown in the latter part of the figure. It may further be concluded that the existing electronic density in the F2-layer is such that the wave length used on this occasion corresponds to the maximum usable frequency for transmission between Delhi and Benares. Evidently then the occurrence of such patterns of periodic fading on a particular frequency indicates the inadequacy of electrons in the ionosphere. As a consequence, it has been found on many occasions that after occurrence of such periodic fading, the signals have disappeared due to scarcity of electrons in the ionosphere. It may, however, be pointed out that the morning disappearance of the signal is due to a cause different from that of the evening disappearance, which we have published earlier (8). It may further be pointed that the variation of the thickness and magnetic field at the ionosphere have been found to affect the magneto-ionic fading considerably. In other words, the observation of such fading pattern may be employed for the study of the variation of the thickness of the ionospheric layer and the magentic field thereon.

5. Slow Periodic Superimposed with Ripples.—The theory of formation of this type of fading has been published earlier. Fig. **5** represents one of the many fading patterns of this type

Fig. IV

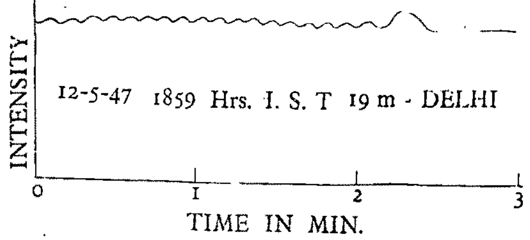


Fig. V

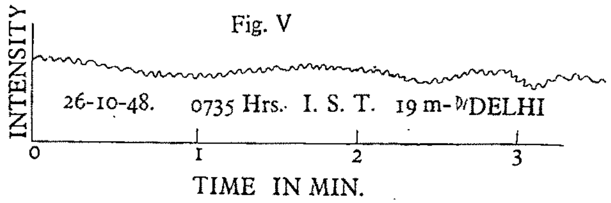
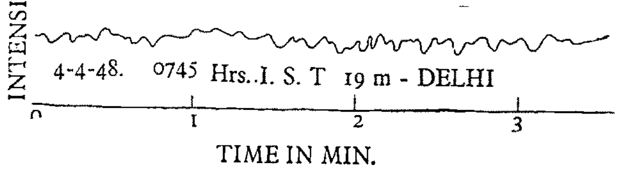


Fig. VI



recorded by us. It will be seen from column 4 of table I that the electronic concentration in the region of the ionosphere at the time of observation was $1{\cdot}8 \times 10^6$ electrons/c.c., which is nearly just enough for double reflection on the wave on 19 metre band. It may be noted that due to the movement the ionospheric layer, the pattern is superimposed with fine periodic ripples. The rate of movement of the layer calculated from this fading pattern was 10·8 km. per hour. Thus it may be concluded that the occurrence of such fading pattern is indicative of fairly high electronic density in the ionosphere for the frequency of transmission used between the transmitting and receiving stations and also the vertical movement of the ionospheric layer. It may be remarked that the appearance of such fading patterns on a particular frequency indicates fairly satisfactory condition of the ionosphere for radio-communication on that frequency.

6. COMPLEX PERIODIC.—It will be observed from table I and Fig. 6 that the formation of complex periodic fading occurs when the electronic density is just enough for single reflection. It may be pointed out that this type is only a special case of magneto-ionic fading and is formed when multiple reflections occur due to lower and upper ordinary or pederson rays and lower and upper extraordinary rays.

It may be observed that the appearance of such fading patterns on a particular frequency ensures that the frequency employed in transmission for the region of reception is very near the maximum usable frequency between the transmitter and the receiver, and as such, it indicates the unsatisfactory condition of the ionosphere for establishing radio-communication between the two stations concerned. This fact has been corroborated by our experimental results.

CONCLUSIONS.—Each type of fading pattern is indicative of a particular state of electronic density in the ionosphere, from where the radio-signal is being reflected. Some of the fading patterns predict the vertical movement of the ionospheric layer as well. Thickness of the ionosphere layers and magnetic field also influence the formation of fading patterns. Fading patterns have also been employed for the calculation of the rate of movement of the ionosphere layer, with an accuracy unapproached by other sensitive methods. The method of calculation of the rate of vertical movement of the ionospheric layer will be communicated separately.

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REFERENCES

1. Banerjee, S. S. & G. C. Mukerjee. 1946. *Science and Culture,* **11** : 571.
2. Banerjee, S. S. & G. C. Mukerjee. 1946. *Nature,* **158** : 413.
3. Banerjee, S. S. & R. N. Singh, 1948, *Indian Physics,* **22**, No. 9.
4. Banerjee, S. S. & R. N. Singh. 1949. *Science and Culture,* **14** : 293-295.
5. Ratcliffe, J. A. & J. L. Pawsey. 1933. *Proc. Camb. Phil. Soc.,* **29** : 301.
6. Pawsey, J. L. 1935. *Proc. Camb. Phil. Soc.,* **31** : 125.
7. Appleton, E. V. & W. J. G. Beynon. 1947. *Proc. Phys. Soc.,* 58, 59.
8. Banerjee, S. S. & R. N. Singh. 1949 *Nature,* **164** : 925.

CONDENSATION OF ALDEHYDES WITH PHENYLACETIC ACID

PART I.—CONDENSATION OF BENZALDEHYDE, SALICYLALDEHYDE AND ANISALDEHYDE WITH PHENYLACETIC ACID

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Perkin's reaction was probably first used by him in 1868 for the preparation of the valuable perfume coumarin by heating salicylaldehyde with sodium acetate and acetic anhydride.[37] Although cinnamic acid had actually been prepared by Bertagini[4] by heating benzaldehyde with sodium acetate and acetyl chloride in a sealed tube at 120-30°, and by Schiff (1870)[41] also by heating benzaldehyde with acetic acid and hydrochloric acid or zinc chloride in a sealed tube at 110-20°, it is Perkin's method, which after modifications from time to time, has survived and attained a classical status by its very wide application in synthetic organic chemistry.

For some time there was a controversy regarding the mechanism of Perkin's reaction. Perkin had suggested that the condensation reaction took place between the aldehyde and the anhydride employed, and the sodium salt acted merely as a catalyst.[38,39] Fittig[8,10] challenged Perkin's view and contended that the salt and not the anhydride condensed with the aldehyde. In the course of this controversy, Stuart[43,44,45] (then working in Fittig's laboratory) made the discovery that the condensation of benzaldehyde to cinnamic acid took place equally well by using sodium malonate in place of sodium acetate, and this lent support to Fitting's theory, generally accepted up to 1928. Although Stuart's findings were contradicted by Breslow and Heausr[6], who finally closed the controversy in favour of Perkin's view, the credit goes to the former for introducing malonic acid as one of the reactants in the condensation in place of acetic acid. Stuart also showed that instead of using acetic anhydride at

180° (in Perkin's reaction), if glacial acetic acid was used at 110°, better results were obtained. This method was then used by many workers, such as Komnenos[24], Stuart[45], Fittig and Mackenzine[9] and others, Michael[25] was able to obtain satisfactory yields by heating the aldehyde with malonic acid alone in a sealed tube for several hours at 140°.

The next important change, so closely associated with the name of Knoevenagel [17], [19], was the introduction of a base, organic or inorganic, as a condensing agent, in place of acetic acid. The inorganic base used was ammonia, in the form of alcoholic ammonia or ammonium malonate or chloride. The organic bases were either the primary bases like aniline and ethylamine, or the secondary ones like diethylamine and piperidine, or its hydrochloride. The inorganic and the primary organic bases were used in full molecular or even larger proportions, while the secondary ones were employed in small proportions only ; thus "piperidine in a few drops" has come to be known as "Knoevenagel's Reagent", and is now a very useful ally of the synthetic organic chemist. Knoevenagel's work extended far beyond the simple aldehyde-malonic acid condensations and several other compounds containing the reactive methylene group, such as malonic ester, acetoacetic ester, cyanacetic acid and ester etc., were also used [15,23].

It is remarkable that the use of pyridine or any other tertiary base escaped the notice of Knoevenagel, although Verley[46], according to Boxer and Linstead[5], was the first to use it (1899), and later on Doebner [6a] and others also used it. A very remarkable observation is due to Harding and Weizmann[13] who specially recommended the use of a tertiary base, like pyridine in cases like the preparation of nonylenic acid from heptaldehyde, where Perkin's method gave a very poor yield of a mixed product and where Knoevenagel's bases too gave poor yield of mixed products. In these condensations, pyridine was generally used in full molecular proportions, or in even greater excess. Staudinger[42] recommended the use of anhydrous pyridine in dry ethereal solution. Florence[11] recommended this method for preparing α-β- unsaturated acids.

In 1924 Robinson recommended to Perkin (Jun.) to use a combination of pyridine with piperidine as a condensing agent, contrary to the then prevailing practice of using the bases singly. Thus Haworth, Perkin and Rankin [14] succeeded in obtaining almost quantitative yield (85%) of 3 : 4-methylenedioxycinnamic acid by using piperonal, malonic acid and pyridine in 1:2·5:6 mol. proportions with a small amount of piperidine, whereas by using Perkin's method they obtained not more than 30% yield. According to the method established by Robinson and Shinoda [40] the proportion of pyridine now used is about 3·5 mols. for one of the aldehyde.

The role of pyridine as a catalytic agent had been a subject of speculation for years. It was in 1928 when Bacharach and Brogan[3] for the first time referred to the catalytic property of pyridine in their report that a few drops of pyridine in Perkin's synthesis of cinnamic acid increased the yield to an optimum value of 85%. In 1931 Boxer and Linstead[5], in their careful survey, expressed the opinion that "tertiary bases as such have a definite catalytic activity", and emphasized this property of pyridine.

The use of molecular proportion of pyridine as a condensing agent failed to effect condensation of salicylaldehyde with malonic acid (Pandya and Surange[31]). Dutt[7] met the same fate even by using a pyridine-piperidine mixture. Yet condensation of salicylaldehyde by other methods, namely Perkin's[38], Stuart's[45], and Knoevenagel's[18] had lon been recorded in the literature. This led Kurien and Pandya [28] to reinvestigate the problem of the condensation of hydroxybenzaldehydes and the use of only 0·15 mol. of pyridine instead of one whole molecule was found to effect condensation. Only a trace of pyridine was thus found to be quite an efficient catalyst. Pandya and co-workers have amply demonstrated the efficacy of 'pyridine-trace' as a catalyst.

It would thus appear that the main factors affecting condensation are (i) the nature of the base as a catalyst (ii) the nature of the aldehyde and the substituent groups present on the benzene nucleus in the case of aromatic aldehydes and (iii) the nature of the substance containing the reactive methylene group. Besides these there are other minor factors such as temperature, time, concentration and solvent.

Besides pyridine, other organic bases have also been tried as catalysts, partly to study their action, and partly to see their comparative efficacy in relation to pyridine. These include piperidine, lutidine, quinoline, etc., and have been studied by Khan, Kurien and Pandya[27]; Kurien, Peter and Pandya[29]; and Vahidy and Pandya.[32-36] The results show that no general rule can be laid down about the relative activity of these bases in condensation reactions. Thus in malonic acid condensations with salicylaldehyde methylacridine gave the highest yield[27], with piperonal piperidine[32], with anisaldehyde isoquinoline[33], and with m-hydroxybenzaldehyde pyridine.[35]

In the present investigations phenylacetic acid has been used in place of malonic acid as a substance containing the reactive methylene group. The aldehydes used are benzaldehyde, salicylaldehyde and anisaldehyde, and pyridine and piperidine, singly as well as in mixtures, have been used as catalysts. Contrary to observations made in malonic acid condensations, pyridine either in traces or even in larger molecular proportions, has been found to have almost failed in condensations with phenylacetic acid. Piperidine in 0·15 mol. proportion has worked very well with all the three aldehydes. In the case salicylaldehyde the maximum yield of the condensation product (phenyl-coumarin) was 63% by using piperidine (0·15 mol.) at 140°, whereas the use of pyridine gave only 14% yield at 160°. Pyridine met the same fate in anisaldehyde-phenylacetic acid condensations when only 6·2% yield of the product was obtained after 36 hours of heating, while piperidine (0·15 mol.), on the other hand, gave 68·5% of α-phenyl (p-methoxycinnamic) acid. Benzaldehyde too refused to condense with phenylacetic acid in presence of pyridine, whereas by using piperidine in traces a yield of 63·3% of phenylcinnamic acid was obtained under optimum conditions. It remains to be seen whether methylacridine and isoquinoline, which of all the bases gave the highest yields in salicylaldehyde-malonic acid and anisaldehyde-malonic acid condensations respectively, will fare similarly in condesations of these aldehydes with phenylacetic acid.

It is noteworthy that in the absence of a condensing agent none of the three aldehydes, under investigation, condensed with phenylacetic acid, although in condensations with malonic acid (in the absence of a condensing agent) benzaldehyde gave 78% of benzalmalonic acid[30], anisaldehyde gave up to 90% of a mixed product[33], and salicylaldehyde gave up to 60% of coumarin-carboxylic acid.

The above results show that the replacement of one carboxyl group in malonic acid by a phenyl group has a retarding effect on the course of condensation and weakens the activity of the reactive methylene group as all the three aldehydes, under reference, give much higher yields of condensation products with malonic acid. The conclusions reached by other workers about the retarding influence of an ortho-hydroxy group and the accelerating effect of a para-methoxy group on the course of condensations have been confirmed by these investigations.

EXPERIMENTAL

(i) *Condensation of benzaldehyde*

(a) *In presence of pyridine.* 1·06 gm. of freshly distilled benzaldehyde and 1·36 gm. of phenylacetic acid (1 : 1 mol.) were mixed together in a 50 c.c. round bottom flask. Varying proportions of pyridine, ranging from 0·15 mol to 6 mols., were then added in different experiments, and temperatures between 100 and 180° were employed. After allowing the reaction mixture to stand overnight and extracting in the usual manner, original aldehyde was recovered and condensation had not taken place.

(b) *In presence of piperidine.* The same amounts of benzaldehyde and phenylacetic acid were heated, using piperidine as a catalyst. From the various experiments performed, in which the proportion of the base taken was 0·15, 1·0, or 3 mols., the temperature ranged between 100° and 180°, and the time of heating was 12, 18, or 24 hours, the optimum conditions were when the aldehyde, phenylacetic acid and piperidine were taken in the molecular proportion of 1 : 1 : 0·15, and the reaction-mixture was heated at 120-25° for 18 hours. Higher temperatures reduced the yield. After leaving the reaction-mixture overnight, it was treated with a 10% solution of sodium bicarbonate and warmed. The solution was extracted with ether to remove the unreacted aldehyde. The aqueous solution on being acidified with hydrochloric acid, precipitated the condensation product. On recrystallisation with hot benzene, white crystals of ∝-phenylcinnamic acid, sharply melting at 170°, were obtained. Melting point given in the literature : Heilbron's Dictionary of Organic Chemistry gives 172°; Walter and Wetzlich, J. Prakt. Chem. 1900 (2), 61,181, gives 170° . Yield 1.37 gm. *i.e.* 63·3% of the theoretical. Equivalent weight found 223 2 ; phenylcinnamic acid, $C_{15}H_{12}O_2$, requires 224·0.

No condensation was found to take place in the absence of any condensing agent even by heating up to 36 hours. Robinson and Shinoda's[40] and Fritz Vorsatz's [12] methods, as well as the use of sodium ethoxide and glacial acetic said, separately, as condensing agents failed to effect condensation.

(ii) *Condensation of anisaldehyde*

(a) *In presence of pyridine.* Pyridine in lower as well as higher proportions and even in combination with piperidine (Robinson and Shinoda's[40], and Fritz Vorsatz's[12] methods) almost failed to effect condensation. A very low yield (6·2%) of ∝-phenyl (p-methoxycinnamic) acid was obtained by using 0·15 mol. of pyridine and heating the reaction-mixture at 170-75° for 36 hours.

(b) *In presence of piperidine.* By using piperidine as a condensing agent, maximum yield (68·5%) of ∝-phenyl (p-methoxycinnamic) acid was obtained when 1·36 gm. of phenylacetic acid, 1·36 gm. of anisaldehyde and four drops of piperidine (1 : 1 : 0·15 mol.) were heated at 140-45° for 18 hours. Longer or shorter hours of heating or the use of higher or lower temperatures reduced the yield. After standing overnight, the condensation product was extracted in the usual manner. After recrystallisation from hot benzene white crystals of the product were obtained, which melted at 189° Literature gives M.P. as 188-89°. (Oglialoro G. 9,533 ; J, 1879, 731). Equivalent weight by alkali-titration method was found to be 254·6 ; ∝-phenyl (p-methoxycinnamic) acid, $C_{16}H_{14}O_3$, requires 254·0.

The use of glacial acetic acid or sodium ethoxide as a condensing agent failed to effect condensation, nor did it take place in absence of a condensing agent.

(iii) *Condensation of salicylaldehyde*

(a) *In presence of pyridine.* Pyridine in this case also did not fare well. At the most an yield of 14% of phenylcoumarin was obtained by heating the aldehyde, phenylacetic acid and pyridine (1 : 1 : 0·15 mol.) at 160° for 18 hours. Even in combination with piperidine there was failure (Robinson and Shinoda's[40], and Vorsatz's[12] methods).

(b) *In presence of piperidine.* 1·22 gm. of salicylaldehyde, 1·36 gm. of phenylacetic acid and 4 drops of piperidine (1 : 1 : 0·15 mol.) were heated at 140° for 18 hours. After standing overnight, the product was treated with 10% sodium bicarbonate solution and then with ether. There remained an insoluble portion, which was separated by filtration. The filtrate on acidification with hydrochloric acid gave some unreacted upon phenylacetic acid. The insoluble residue was once more washed with water and ether. On recrystallisation from glacial acetic acid, a white substance, sharply melting at 138°, was obtained. It did not give an effervescence with sodium bicarbonate and had no effect on blue litmus paper. The substance was identified as phenylcoumarin. Literature gives melting point of phenylcoumarin as 139-40° (Oglialoro G 9, 428 ; B. 12,2367 ; J. 1879, 731 ; Walther and Wetzlich, J. Prakt. chem., (2). 61).

The use of glacial acetic acid or sodium ethoxide could not induce condensation ; nor did the condensation take place in the absence of a condensing agent. Fritz Vorsatz's[12], as well as Robinson and Shinoda's[40] methods also failed.

SUMMARY

(i) Benzaldehyde, salicylaldehyde and anisaldehyde have been condensed with phenylacetic acid.

(ii) Pyridine has not been successful as a catalyst in these condensations, whereas piperidine has turned out to be effective.

(iii) The reactive methylene group has proved to be less active in phenylacetic acid than in malonic acid.

(iv) The group effect of ortho-hydroxy and para-methoxy groups is in line with earlier findings.

References

1. Annual Reports, Chemical Society 1939. **36** : 210.
2. Armstrong, H.E. 1933. *J. Chem. Soc.*, 470.
3. Bacharach and Brogan. 1928. *J. Amer. Chem. Soc.*, **50** : 3334.
4. Bertagini, 1856. *Annalen der chemie*, **100** : 126.
5. Boxer and Linstead. 1931. *J. Chem. Soc.*, 738-40.
6. Breslow and Hauser. 1939. *A. Amer. chem. Soc.*, **61** : 786.

6 a. Doebner. 1900. *Berich. Chem.*, **33** : 2140 ; 1901, *ibid.*, **34** : 53 ; 1902. **35** : 1136, 2137.

7. Dutt, S. 1925. *J. Indian. chem. Soc.*, **1** : 297.
8. Fitting. 1881. Ber., **14**. 1824. *Annalen der chemie*, 1885, **227** : 48.
9. Fitting and Mackenzie. 1894. *Annalen der chemie*, **283** : 82.
10. Fitting and Slocum. 1885. *Annalen der chemie*, **227** : 61.
11. Florence, G. 1927. *Bull. Soc. Chem.*, (iv), 41, 440.
12. Fritz Vorsatz. 1936. *J. Prakt. Chem.*, **145** : 265.
13. Harding and Weizmann. 1910. *J. Chem. Soc.*, **97** : 299.
14. Haworth, Perkin (Jun) and Rankin. 1924. *J. Chem. Soc.*, **125** : 1693.
15. Cnoevenagol 1896, *German Patents*, 97735.
16. ,, 1902, *German Patents*, 156560.
17. ,, 1903, *German Patents*, 161171.
18. ,, 1898, *Berich. der chemie*, **31** : 2585, 2593.
19. ,, 1898, *Ibid*, **31** : 2596.
20. ,, 1896, *Ibid*, **31** : 2768.
21. ,, 1902, *Ibid*, **35** : 395.
22. ,, and Gross 1898, *Ibid*, **31** : 2594.
23. ,, and Schroter. 1904. Ber.*der chemie*, **37** : 4487.
24. Komnenos. 1883. *Annalen der chemie*, **216** : 115
25. Michael, A. 1884. *J. chem. Soe.*, **5** : 205
26. Pandya, K.C. *et al.* 1937. *Proc. Indian Acad. Sci.*, **6** : 181 ; 1938. *ibid.*, **7** : 381 ; 1939. *ibid.*, **9** : 508 ; 1939 ; *ibid.*, **9** : 511.
27. Pandya, Khan, and Kurien 1935. *Proc. Indian Acad. Sci.*, **1** : 440 .
28. ,, and Kurien. 1984, *J. Indian chem. Soc.*, **11** : 825
29. ,, Kurien, and Peter 1935, *Proc. Indian Acad. Sci.*, **1** : 775.
30. ,, and Miss Pandya 1941, *Ibid*, **14** : 112.
31. ,, and Surange. 1934. *J. Indian chem. Soc.*, **11** : 824.
32. ,, and Vahidy. 1935. *Proc. Indian Acad. Sci.*, **2** : 402.
33. ,, ,, 1936. ,, **4** : 134.
34. ,, ,, 1936. ,, **4** : 140.
35. ,, ,, 1936. ,, **4** : 144.
36. ,, ,, 1937. ,, **5** : 437.
37. Perkin (Sen.), W.H. 1868. *J. chem. Soc.*, **21** : 53.
38. ,, 1887. *Ibid.* **32** : 388.
39. ,, 1877. *Jahresber. Fortschr. chem.*, 789.
40. Robinson and Shinoda. 1925. *J. chem. Soc.* **127** : 1977.
41. Schiff. 1870. *Berich. Chem.*: **3** : 414.
42. Staudinger Wayl's "Methods of Organic Analysis".
43. Stuart, C.M. 1883. *J. chem. Soc.*, **43** : 403.
44. ,, 1883. *Berich. chem.*, **16** : 1436.
45. ,, 1886. *J. chem. Soc.*, **49** : 366.
46. Verley, A. 1899. *Bull. Soc. Chem.*, **21** : 143 414.

MUTA-ROTATION OF d-OXYMETHYLENE CAMPHOR AND ITS CONDENSATION PRODUCTS WITH PRIMARY AMINES AND OF d-OXYMETHYLENE CARVONE

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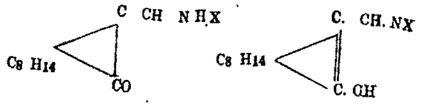
Pope and Read[1] and Bishop, Claisen and Sinclair[2] have observed that d-Oxymethylene camphor and its derivatives show a change in optical rotation with respect to time.

In an earlier paper[3], I have shown that the change of d-Oxymethylene camphor in alcohol is represented as :



In this paper this work has been extended to various other solvents and to its derivatives with primary amines, prepared as described by Bishop, Claisen and Sinclair.

d-Oxymethylene camphor. Patterson[4] and Winter[5] showed that the optical activity of a substance is influenced by temperature. d-Oxymethylene camphor behaves similarly. The change is not due to muta-rotation, because on cooling quickly to the original temperture, the same original rotation is observed. Moreover, the substance shows this change even when muta-rotation change is complete after 48 hours.

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[1] *J. chem. Soc.*, **94** ; 176 (1909).

[2] *Annalen der chemie*, **28** : 331 (1894)

[3] *J. Indian Chem. Soc.*, **70** : 545 (1940).

[4] *J. chem. Soc.*, **93** : 1894 (1908).

[5] *Z. Physik. Chem.*, **41** : 176 (1920).

TABLE 1

Solvent : Absolute alcohol — Length of the tube : 1 decimetre

Wt. of the substance in 10 c. cs.	Temp.	(α) d	(α) D	Remarks
0·2518 gms.	25°	4·85	192.6	Before muta-rotation change is complete.
,, ,,	35°	4·65	184.6	,, ,,
0·1878 ,,	0°	3·85	205.0	,, ,,
,, ,,	10°	3·80	202.6	,, ,,
,, ,,	27°·5	3·70	197.3	,, ,,
,, ,,	35°	3·50	186.7	,, ,,
,, ,,	55°	3·30	176.0	,, ,,
0·2454 ,,	25°	4·40	183.4	,, ,,
,, ,,	40°	4·40	179.3	After muta-rotation change is complete
,, ,,	55°·0	4·30	175.3	

TABLE 2

Solvent : Acetone — Length of the tube : 1/2 decimetre

Wt. of the substance in 10. c. cs.	Temp. in °C	(α) d	(α) D	Remarks
0. 4870 gms.	35°	3·30	135·5	Crude sample
,, ,,	30°	3·35	137·6	,,
,, ,,	23·8°	3·45	141·7	,,
,, ,,	10°	3·60	147·9	,,
0. 3944 ,,	23·5°	2·83	143·0	Crystallised.
,, ,,	10°	3·05	154·7	,,

Walden[6] and Seirtsema[7] observed that optical rotation is a function of the nature of the solvent. The results with d-oxymethylene camphor are as follows :

TABLE 3

Solvent	Wt. of the substance in 100 c. cs.	(α) d in 2 d. cm. tube	(α) D.
Alcohol	2·1896 gms.	8·30	190·0
,,	2·6312 ,,	10·10	191·0
Benzene	2·1208 ,,	5·80	140·0
,,	0·8848 ,,	2·55	144·2
Acetone	4·8700 ,,	13·80	181·0
,,	3·9440 ,,	11·20	141·9
Methyl alcohol	0·9756 ,,	3·75	192·0
,,	0·9392 ,,	3·50	187·0

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[6] *Berichte der Chemie*, **38** : 345 (1905).

[7] *Arch. Neerl Sci.*, **11** : 2, 29 (1892).

The above results, unlike the observations of Schmeider[8] Malosse[9] Oudemann[10] and Marshall[11] show that the optical rotation of d-oxymethylene camphor does not change with its concentration.

I have shown that d-oxymethylene camphor shows muta-rotation in alcohol. The same thing is observed in case of benzene and other solvents.

TABLE 5

Temp.	Wt. in 100 c.cs.	(a) d	Time	Length of the tube	Solvent	Ratio of $\frac{(\alpha)\,d_o}{(\alpha)\,d_t}$
21·2°C.	2·1208 gms	5·80	0 hrs	2 d. cm.	Benzene	...
,,	,, ,,	3·50	6 ,,	,, ,,	,,	0·0603
23·4°C.	0·884S ,,	2·55	0 ,,	,, ,,	,,	...
,, ,,	,, ,,	1·55	6 ,,	,, ,,	,,	0·0601
...	1·5340 ,,	1·30	0 ,,	1/2 ,,	Acetone	...
...	,, ,,	1·20	21 ,,	,, ,,	,,	0·9020
23 8°	4·8700 ,,	3·45	0 ,,	,, ,,	,,	...
20°C.	,, ,,	3·20	21 ,,	,, ,,	,,	0·9280
...	0·975 ,,	3·80	0	2 ,,	Methyl alcohol	
19·6°C	,, ,,	2·70	21 ,,	,, ,,	,,	0·7000
...	0·9392 ,,	3·40	0 ,,	,, ,,	,,	...
...	,, ,,	2·35	21 ,,	,, ,,	,,	0·6980

The following results show that the muta-rotation change d-oxymethylene camphor in benzene is unimolecular.

0·8848 gms. in 100 c.cs. in 2 decimetre tube. Temp. 23° C.

T (Time) in minutes	(α) d	a — x	$K_1 \times 2·30$
0	2·55	1·0 (a)	—
8	2·20	0·63	0·0234
16	1·95	0·40	0·0243
25	1·80	0·25	0·0241
—	1·65	0·10	0·0270
37	1·60	0·05	0·0260
50	1·55	—	—
60	1·55		
—		—	—

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[8] *Annalen der Chemie*, **207** : 257 (1881).
[9] *Compt. rend.*. **153** : 56 (1911).
[10] *Annalen der Chemie*, **182** : 46 (1876).
[11] *J. chem. Soc.*, **63** : 227 (1893).

Anilino-d-methylene camphor ; B-Napthylamino-d-methylene camphor ; -Para Toludino-d-methylene camphor :—

The compounds were prepared as given by Pope and Read[12] and were crystallised from solvents mentioned by them. The compounds obtained by me show the same melting points as given by Pope and Read.

Pope and Read have observed that anilino-d-methylene camphor shows muta-rotation in benzene solution. Their results indicate that muta-rotation attains a constant value and it changes from (∝) D 309·4 to 273·1. It is interesting to observe that their initial value of muta-rotation for different concentrations of the substance is not the same. It seems therefore that the value of specific rotation is a factor of concentration for this compound. The two concentrations at which I have determined the specific rotation for this substance also show the same variation.

Weight in 25 c. cs. of benzene	(∝) d in 2 d. cm. tube	(∝) D.
0·2134 gms.	5·0	293
0·1002 ,,	2·45	304

I have studied the rate of muta-rotation change.

Wt. of the substance, 0·1002 gms. in 25 c. cs. Solvent : Benzene, Temp : 22·8°C.

Time (mins.)	(∝) D.	(∝) D.	$\frac{1}{t} \log \frac{a}{a-x} = K_1 \times 2{\cdot}3$
0	2·45	305·0	0·00123
30	2·25	280·0	0·00153
100	1·90	237·0	0·00110
215	1·15	143·0	0·00134
510	0·40	50·0	0·00153
50 hours	0·10	12·5	...

The results indicate that (∝) D changes from 305 to 1·25 unlike the results of Pope and Read[13] and that the change is unimolecular.

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[12] *Ibid.*, 177-178 (1909).

[13] *J. chem. Soc.*, **94** : 176 (1909).

Para Toludino-d-methylene camphor

Pope and Read (*loc. cit.*) have observed that the specific rotation of this compound is 281·8 in benzene and changes to 246·5 after 90 minutes. My results indicate that the value is as high as 336 and finally changes to zero.

Wt. of the substance in 25 c. cs. of benzene in decimetre tube.	
0·0760	336
0 0740	304

The change of rotation with time is given below :—

Wt. of the substance 0·0740 gms. in 25 c. cs. Temp. 22°C.

Time (mins)	(α) D	$\frac{1}{t} \log \frac{a}{a-x} = K_1 \times 2\ 30$
0	304·0	
30	269·0	0·00171
95	202·0	0·00175
165	127·0	0·00230
215	85·0	0·00258
290	51·0	0·00268
380	26·0	0·00284
540	00·0	–
50 hours	00·00	–

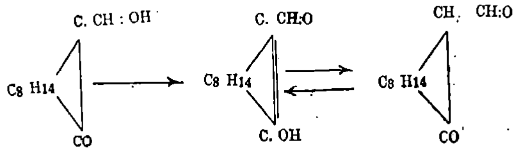
B-Napthylamino d-methlene camphor :—

Pope and Read (*loc. cit.*) observed that the value of specific rotation is 264·6 and falls to 10·1 after 24 hours. My observations show that this value is as high as 277 and falls to zero after fifty hours.

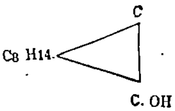
Wt. of the substance 0·1037 in 25 c. cs. Temp. 22°C.

Time (Mins)	(α) D.	$\frac{1}{t} - \frac{a}{a-x} = L_1 \times 2{\cdot}30$
0	277·0	–
30	259·0	0·0098
70	241·0	0·0087
100	229·0	0·0083
140	204·0	0·0093
185	186·0	0·0100
260	156·0	0·0095
50 hours	0	– –

The above determination of velocity of muta-rotation of the condensation products of d-oxymethylene camphor with the primary amino compounds, aniline, p-toludine and p-napthylamine clearly shows that the change is a simple monomolecular reaction of the type A——› B. Pope and Read (*loc. cit.*) suggest that isodynamic change is of the type :

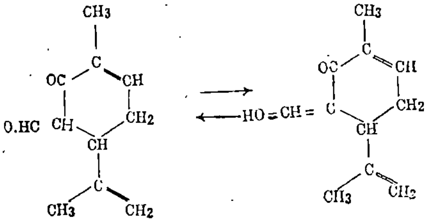


In view of the fact that d-oxymethylene camphor is mainly enolic, the derivative should have the constitution CH : NX; and



the isodynamic change should be a change from enol to keto form like the muta-rotation change of *d-oxymethylene camphor.*

D-Oxymethylene carvone. It was prepared by adding amyl formate to a mixture of molecular sodium and carvone. using anhydrous ether as medium, d-oxymethylene carvone can show isodynamic isomerism and if it is a mixture of the two forms, it should be possible to shift the equilibrium by adding suitable substance and the shift can be studied by observing the change in specific rotation of the original compound.



The alcoholic solution of d-oxymethylene carvone shows no change in rotation with time, but the addition of hydrochloric acid causes a marked effect and the value becomes constant after 24 hours. It is further observed that the reaction proceeds to the same extent as is shown by the constancy of the ratio of (∝) d when $t=0$ and $t=\infty$ for different original concentrations of d-oxymethylene carvone.

Vol. of alcohol 25 c. c 0·4 c.c; of 2·117 N HCl added.

Wt. of the substance	(∝) d t=0	(∝) dt= ∞	$\frac{(\propto)\ d\ t=\infty}{(\propto)\ d\ t=0}$
0·5038 gms.	0·40	0·95	2·37
1·2900 ,,	1·7	4·05	2·38

Constancy of these results however does not show that the change is an equilibrium reaction, since results would be obtained if the change is unimolecular of the type A—B and goes to completion. In this case unimolecular velocity constant should be obtained. The following results illustrate this (Temp. 22·8°C)

Wt. of the substance in 25 c. c. of alchool.	Amount cf 2·117 N HCE	t (in mins.)	(∝) d	a-x	$\frac{1}{t}\log\frac{a}{a-x}=K_1+2{\cdot}3$
0·5038	0·2 cc.	0	0·40	0·55	...
		28	0·55	0·40	0.0049
		48	0·60	0·35	0·0041
		88	0·70	0·25	0·0040
		108	0·75	0·20	0·0040
		193	0·85	0·10	0·0038
		∞	0·95	...	...
0·5038	0·4 cc.	0	0·40	0·55	...
		12	0·50	0·45	0·0072
		27	0·60	0·35	0·0072
		41	0·65	0·35	0·0066
		54	0·70	0·25	0·0064
		∞	0·95	...	...
1·29	0·4 c. c.	0	1·70	...	...
		23	2·40	1·65	0·0066
		44	2·80	1·25	0·0063
		66	3·10	0·95	0·0060
		82	3·30	0·75	0·0060
		104	3·50	0·55	0·0060
		134	3·70	0·35	0·0061
		170	3·85	0·20	0·0063
		8	4·05	...	...

The results indicate that although the concentration of HCl is doubled, the velocity constant is not doubled. It seem therefore the

the reaction is one-sided and is accelerated by H-ions, and the oxy-methylene carvone is not a mixture of the two isomers.

My thanks are due to Dr. S. S. Deshapande and Dr. W. V. Bhagwat, for their interest in this work.

SUMMARY

(i) Optical rotation of d-oxymethylene camphor changes with temperature, nature of the solvent but not with concentration. It shows muta-rotation in benzene, acetone, methyl alcohol and ethyl alcohol and the change is unimolecular in all solvents.

(ii) The optical rotation of anilino-d-methylene camphor, toludino-d-methylene camphor and............ ......*B* Napthyl-amino-d-methylene camphor changes with time and the change is...............uimolecular. The rotation changes to zero in the last two cases.

(iii) Optical rotation of d-oxymethylene carvone changes when HCl is added and the change is unimolecular.

HYDROGEN BONDING AND RESONANCE FROM THE STUDY OF PARACHOR

By W. V. BHAGWAT, R. P. SHUKLA AND KHANDEKAR,
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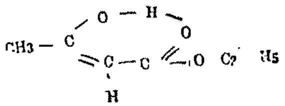
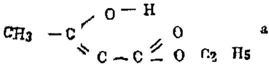
Bhagwat and R.P. Shukla[2] for the first time showed that there is a relationship between parachor and resonance. They discussed the conditions for resonance on the basis of parachor and concluded that this should generally occur when the various structures have the same or practically the same calculated parachor. The observed parachor should however tend to decrease, as the interchangeable electrons should pass more time in the vicinity of the nuclei, for then alone the potential energy tends to be minimum, a condition necessary for resonance. The low parachor values for aliphatic diazocompounds and N_3 group suggested structures, not supported by electron diffraction and other methods. The explanation by Bhagwat and Shukla (*loc. cit.*) that it is due to resonance is now in harmony with the conclusions from other methods.

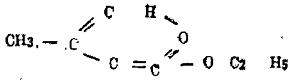
Sidgwick[10] made an important suggestion from his observations on the parachor measurements of 0-substituted phenols, that each increase of one unit in covalency results in an approximate decrease of 12·8 units in parachor. He thus supported the actual formation of a ring by hydrogen bond. In case of aceto-acetic ester we have, for example, the structure :



In another paper[4] he attributed the ring behaviour to resonance :



and

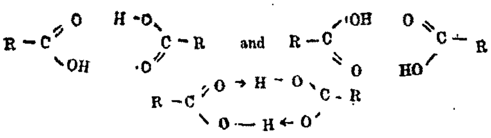


The ring behaviour of B-ketoesters, including ethylacetoacetate is confirmed by Sidgwick[10] from the solubility of enolic form in hydroxyl solvents and in benzene and by Bawn[11] from infra-red spectra.

It will be clear from the above considerations that since parachor decrease can either be due to resonance or due to co-valency increase, it is difficult to settle between the two alternative structures, unless the relative magnitude of parachor decrease in two cases is determined.

The covalency increase that we are considering in this paper is that of hydrogen atom and hence resonating structures that will be considered for comparison will be of similar type ; because the comparison of parachor decrease in two cases must be made under similar conditions. If we consider covalency increase for chlorine atom as in case of molar compounds formed by carbontetrachoride with acetone, ethyl acetate and ether, a resonating structure involving chlorine will have to be compared for parachor decrease due to these two causes.

Carboxylic acids are known to exist as dimers in benzene, chloroform and carbon tetrachloride and as monomers in ether.[3,7,9] Thus resonance structures are only possible for these acids in ether and some other solvents, while in other above solvents, they will exist as dimers containing hydrogen bonds.



The difference in parachor of carboxylic acid in ether and other solvents, if any, will help to establish whether the decrease in parachor is due to resonance or due to actual hydrogen binding. With this object in view we have studied the parachor of carboxylic acids in these solvents. Our results are as follows :—

In calculating the parachor of the solute in solution the mixture law of Hammick and Andrew[5] has been used.

x = molecular fraction of carboxylic acid.
t = Temperature in °C, d = density of the solution.
r = Surface tension of the solution.
Pm = The parachor of the solution and
Px = the parachor of the carboxylic acid.

The results for P^x have been calculated in all cases with double the molecular weight of the acid (dimer).

Acetic acid

Solvent	x	t	d	r	Pm	Px (Acetic acid)
Benzene	0·4278	23·9	0·9634	22·7	228·7	260·5
	0·4466	24·7	0·9594	27·3	229·5	260·0
	0·5838	25·0	0·9964	26·6	237·7	261·0
	0·6358	25·4	0·9888	26·7	240·9	261·2
	0·7034	26·3	0·9997	26·4	244·1	260·5
CCl_4	0·4760	30·5	1·283	24·7	240·4	260·5
	0·4286	30·4	1·307	24·5	237·3	260·1
	0·3825	32·7	1·325	24·6	235·4	260·5
	0·4826	32·0	1·271	23·9	239·4	260·2
	0·5905	32·0	1·214	24·1	242·9	260·3
	0·6766	30·7	1·252	29·7	247·7	260·7
	0·5483	30·7	1·249	25·1	242·5	260·5
$CHCl_3$	0·5894	24·7	1·182	25·8	228·4	260·1
	0·6336	24·7	1·166	26·1	232·1	260·5
	0·7451	24·2	1·137	27·3	240·9	260·8
	0·7258	24·2	1·136	26·3	238·9	260·1
	0·3370	24·2	1·246	22·8	209·1	260·4
	0·2905	24·9	1·310	25·5	205·4	260·1
Ethyl ether	0·5130	19·2	0·0908	28·80	248·8	284·1
	0·4019	19·2	0·8730	26·80	241·6	284·5
	0·3712	19·2	0·8640	26·30	238·7	284·4
	0·2987	19·2	0·8380	24·70	233·4	284·2
	0·3660	18·2	0·8630	26·30	238·2	283·9
	0·4977	18·2	0·9030	29·80	249·9	284·4
	0·5211	18·2	0.9170	29·80	249·7	284·0

2. *Propionic acid*

Solvent	x	t	d	r	Pm	Px(Propionic acid)
Benzene	0·5230	26·3	0·9535	26·10	279.5	343·7
	0·4309	25·7	0·9456	24·14	264.1	343·8
	0·3322	25·7	0·9358	30·26	250·7	342·5
	0·4348	27·6	0·9430	27·90	264·3	341·5
	0·4528	28.0	0·9539	28·91	266·9	341·8
	0·6446	28·4	0·9581	27·09	291·5	341·2
CCl_4	0·33067	31·0	1·2749	44·657	260·19	341·51
	0·36274	32·3	1·2537	44·289	263·80	341·08
	0·37838	31·0	1·2531	42·964	264·42	340·51
	0·39537	31·0	1·2348	41·736	267·82	341·02

	0·42487	31·0	1·2948	40·551	271·14	340·81
	0·43813	31·0	1·2131	40·166	272·63	340·09
	0·44566	30·7	1·2089	40·013	273·95	340·99
	0·45878	30·7	1·2026	34·506	275·25	340·41
$CHCl_3$	0·42248	24·8	1·1948	26·327	249·33	340·02
	0·50865	24·9	1·1487	24·332	259·34	340·99
	0·61193	25·1	1·1153	26·685	279·58	341·00
	0·73552	25·1	1.0681	26·693	298·81	340·52
	0·30020	25·3	1·2642	26·724	230·39	340·71
	0·27970	25·3	1·2803	27·090	226·99	340·29
	0·19183	25·2	1·3231	25 982	213·28	340·81
Ethyl ether	0·3946	18·5	0·8660	27·06	271·2	362·3
	0·3776	18·0	0·8734	28·18	268·7	362·5
	0·4075	18·0	0·8776	27·95	273·0	362·1
	0·2976	18·0	0·8380	25·12	256·5	362·1
	0·3115	18·3	0·8442	26·77	258·7	362·6
	0·2798	18·3	0·8332	24·94	254·0	362·8
	0·2185	18·1	0·8201	24·52	244·73	362·5
	0·1165	18·2	0·7009	23·10	236·6	362·1

3. *Benzoic acid*

Solvent:	x	t	d	r	Pm	Px (Benzoic acid)
Benzene	·02149	29	·8847	28·21	212·71	535·8
	·02471	29	·8851	28·13	213·75	536·3
	·02579	30	·8857	28·31	214·26	537·0
	·02836	26	·8880	28·29	215·02	536·3
	·02966	28	·8758	26·50	215·45	537·6
CCl_4	·02228	35	1·5450	25·33	225·97	536·5
	·02434	26	1·5590	26·37	226·68	537·6
	·02569	28	1·5550	26·23	227·08	536·3
	·02655	25	1·5520	26·04	225·96	536·9
	·02757	28	1·5470	25·84	227·70	536·9
$CHCl_3$	·04482	35	1·4190	26·04	198·90	537·6
	·04649	27	1·4310	26·99	199·51	537·9
	·05929	28	1·4260	27·59	204·05	537·1
	·07065	35	1·4090	27·36	208·16	537·3
	·07610	28	1·4050	26·30	208·04	537·0
CS_2	·009766	37	1·2380	30·37	147·33	537·4
	·011550	37	1·2380	30·31	148·00	537·0
	·01569	33	1·2430	30·93	149·64	538·0
	·01640	29	1·2470	31·13	149·44	538·8
	·01841	29	1·2420	31·19	150·83	539·5
Ethyl acetate	·03602	29	·9061	23·61	227·45	548·9
	·06716	40	·9055	22·95	238·68	549·9
	·07941	35	·9251	25·37	242·21	549·2
	·09491	30	·9410	26·28	247·31	549·1
	·12300	30	·9464	26·37	256·69	549·3

Acetone	·01848	29	·8054	24·14	169·15	549·1
	·04021	35	·8237	24·86	177·58	549·1
	·04840	40	·8225	24·98	180·79	549·4
	·09333	40	·8708	27·36	198·20	549·3
	·09649	30	·8746	27·19	199·13	547·6
Ethyl ether	·02284	29	·7444	38·26	260·75	549·4
	·04625	25	·7681	38·84	267·70	549·2
	·07071	25	·7891	40·22	274·82	548·9
	·08688	25	·8071	40·43	279·53	548·3
	·08954	25	·8042	40·62	280·36	549·1
Acetic acid	·01971	29	1·0530	30·09	141·580	547 0
	·03404	29	1·0600	31·05	147·660	548·4
	·05440	29	1·0670	32·32	155·820	550·8
	·06567	35	1·0640	31·56	160·940	550·2
	·07660	30	1·0740	32·94	165·370	549·3

4. Butyric acid

Solvent	x	t	d	r	Pm	Px (Butyric acid)
Benzene	0·3644	27·5	0.9228	29·91	287·1	430·1
	0·3214	28·0	0·9183	28·61	277·2	429·8
	0·2711	28·0	0·9166	29·54	266·0	430·2
	0·2182	27·5	0·9198	30·59	254·1	429·8
	0·1855	27·5	0·116	28·74	171·87	430·1
CCl_4	0·3390	31·8	1·260	26·65	291·4	430·5
	0·3526	31·6	1·246	26·42	294·2	430·7
	0·3763	31·7	1·233	26·82	299·5	430·2
	0·41267	31·7	1·2026	26·242	306·91	430·7
	0·42935	32·1	1·2017	27·177	310·31	430·41
	0·3895	32·1	1·2212	26·516	302·42	430 9
	0·43073	32·1	1·1965	26·785	310·86	430·7
	0·43511	32·4	1·1965	27·466	311·78	430·9

5. Salicylic acid

Solvent	x	t	d	r	Pm	Px (Salicylic acid)
Methyl acetate	·05128	27	·9734	27·07	197·75	586·2
	·07224	32	·9845	27·67	206·39	586·8
	·07442	27	·9940	28·63	207·17	586·8
	·08492	27	1·0030	29·95	212·71	589·0
Ethyl alcohol	·05051	28·0	·8826	27·83	152·52	590·4
	·0613	28·0	·8984	28·55	155·91	589·5
	·07950	35·0	·8993	26·34	163·42	590·2
	·09581	28·0	·9173	28·16	171·04	590·1
Ethyl acetate	·05539	31·0	·9462	27·72	236·74	589·3
	·06291	26·3	·9545	27·34	236·42	588·6
	·07602	26·3	·9576	27·29	244·38	89·1
	·09663	26·3	·9649	29·51	256·72	5590·1

The calculated parachor of acetic acid as dimer without hydrogen bonds is 282·2, for propionic acid 360·2 and for benzoic acid 550. The observed results in ether and other monomeric solvents as acetone, acetic acid, ethyl alcohol and ethyl acetate are 284, 362 and 549 respectively. The agreement is fair and it can be concluded that there is no appreciable decrease due to resonance and heteromoleculor hydrogen bond with the solvent, between the molecules of carboxylic acids. On the other hand where homomolecular hydrogen bond is formed, the values for acetic acid, propionic acid and benzoic acid are 260, 340 and 537 respectively. Thus there is an appreciable decrease in parachor due to two hydrogen bonds existing in the dimer of carboxylic acids. The average value for each bond is 12, 11 and 6 as observed. In case of benzoic acid the result is rather low.

In any case the falls are quite convincing so as to distinguish between resonance and hydrogen bond formation. Our results with butyric acid in benzene and carbon tetrachloride give a value of parachor—430, the calculated value being 438. This indicates hydrogen bond formation as expected.

It is interesting to note the parachors of pure liquid formic and acetic acids as obtained experimentally are 93·3 and 133·5 respectively 68. They are known to be associated. The calculated values are 102 and 141. The decrease in values supports hydrogen bonding and association of these acids as supported by electron diffraction methods.

Salicylic acid gives a value of 589 in ethyl acetate and methyl acetate, while the calculated value is 590 for resonating molecules. However, this acid presents a complex structure and further study in necessary.

We conclude therefore that parachor determination can decide between the resonating and hydrogen bond structures if both alternative structures are possible. The observed parachor and calculated one do not differ much in the first case, while in case of hydrogen bond there is a sudden fall in parachor value.

REFERENCES

1. Bawn 1938 in Glasstone's. Recent Advances in Physical Chemistry, p. 51.
2. Bhagawt, W.V. & R.P. Shukla. 1950. *J. Indian Chem. Soc.*, **27** : 283; 1951. **28** : 106.
3. Coolidge, A. S. 1928. *J. American Chem. Soc.*, **50** ; 2166.
4. Glasstone, 1938. Recent Advances in Physical Chemistry, p. 93.
5. Hammic & Andrew. 1929. *J. Chem. Soc.*, p. 754.
6. Hunter & Maass. 1929. *J. American Chem. Soc.*, **51** : 153.
7. Lassettre, E. N. 1937. *Chem. Rev.*, **20** : 259.
8. Mumford & Phillips 1929. *J. Chem. Soc.*, p. 2112.
9. Pauling L. & L. O. Brockway. 1934. *Proc. nat. Acad. Sci.*, **20** : 336.
10. Sidgwick. 1925. *J. Chem. Soc.*, **29** : 909, 2739.

RECENT ADVANCES IN OUR KNOWLEDGE OF FLUORESCENT REAGENTS

By C. N. HAKSAR, M.Sc., PH.D. (BRISTOL), *Department of Chemistry, Victoria College, Gwalior.*

FLUORESCENCE is defined by E. J. Bowen[3] as the "re-emission of absorbed radiation." This phenomenon was observed and recorded hundreds of years ago, for example, Boyle noted the colour of extracts of certain woods. When in 1833, Sir David Brewster[4] allowed a beam of sunlight to pass through an extract of green leaves, little did he suspect the immense field that lay before him. Brewster observed at this early date that the green-coloured constituent of leaves, *viz.* chlorophyll, exhibited a deep reddish "internal dispersion" or *fluorescence,* as it is now called. Little progress was made on the theoretical side until 1852, when Sir George Stokes gave a lucid account of the true nature of fluorescence, when he observed that fluorsparor calcium fluoride fluoresces in ultra-violet light and invented the term *fluorescence* for this type of luminescence.

In the terminology of modern physics, no substance can emit fluorescent light unless it has been previously excited or *quantised* by absorption of radiation. Quantisation deals with the increase in energy by atoms or molecules through intricate electronic, atomic or molecular mechanisms. When an atom or molecule absorbs energy, certain changes occur in the normal movement of the electrons within the atom and in the atom or molecule itself. These energy changes may be given by the Bohr condition:

$$hr = E_2 - E_1$$

where h is Planck's constant, r is the frequency of light and E_2 and E_1 are the energies in the initial and final stages respectively. It will be seen from above that a positive value for r corresponds to absorption, while a negative value corresponds to emission of light.

Fluorescence in Analysis. After a long induction period extending from the beginning of its scientific study in the early nineteenth century, fluorescence has recently attracted interest outside the growing circle of its own devotees and has now been added to the expedients by which chemical reactions can be observed simply and surely, when only small quantities of materials are involved. In fluorescence analysis, the object under investigation is observed while it is being irradiated with ultra-violet light, most conveniently obtained from mercury-vapour lamp, most of the visible rays being removed by means of a suitable glass-filter. Under these conditions it is probable that the majority of organic compounds fluoresce and glow in colours, often characteristic and intense. It is of interest that many compounds, such as quinine sulphate and anthracene, are colourless in ordinary light but may be detected and identified by their characteristic fluorescent colours in ultra-violet light. It must be remembered, however, that fluorescence may be, and often is, due to the presence of minute quantities of impurities. There are several well known examples of this. A highly fluorescent commercial sample of fluorene or carbazole when purified gives non-fluorescent materials. After repeated crystallisations, quenching of fluorescence in certain substances may take place or it may completely disappear as well (10)*.

As far back as 1868, F. Goppelsroder noticed that morin, in the presence of an aluminium compound gave an intense green fluorescence in ultra-violet light. The applications of fluorescence in analysis have since then been very wide. "One of the most striking contributions of organic chemistry to medical science, in recent years, was Cook's isolation and identification of the carcinogenic compound in coaltar (6)." The compound proved to be 3 : 4-benzpyrene, the isolation of which was made possible by its characteristic fluorescence (8). In vitamin research also fluorescence has been successfully employed. When a slice of tissue, fixed in formaldehyde, is observed in filtered ultra-violet light, the presence of vitamin A_1 is revealed by a green fluorescence, while that of vitamin A_2 shows a reddish glow. The capsaicin content of paprika has been determined by titrating with an alcoholic solution of picric acid in ultra-violet light, the end-point being indicated by the disappearance of fluorescence (G. Nogra'dy).

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* The claim that fluorescein loses fluorescence completely by recrystallisation has not been accepted by others.—*Editor.*

FLUORESCENCE IN CHROMATOGRAPHY. In 1906, M. Tswett, the Russian botanist, first used the technique of chromatographic adsorption. Recent researches into the early history of chromatography, however, indicate that the first recorded experiments on capillary analysis should be attributed to R. F. Runge, who in 1850, analysed mixtures of dyes on blotting paper and earlier had suggested spot tests for bleaching solutions, using dyed cotton fabric or paper impregnated with starch and potassium iodide (19). Tswett, by his ingenious method, was able to demonstrate the plurality of chlorophyll and carotene. Between the years 1906 and 1930 little use was made of this method. Since its brilliant application to the isolation and separation of the carotenoid pigments by R. Kuhn (12), the great possibilities of the method as an exceedingly useful separative technique, have been widely appreciated. Although originally the method was only applied in the case of coloured substances, it has now been used in the case of colourless substances as well. This is based on the fact that many colourless substances fluoresce when irradiated with ultra-violet light and thus what may be called [illegible] *ultra-chromatogram* is developed. It should, however, be remembered that ultra-violet light is capable of decomposing compounds, for example, cholesterol, and therefore, long exposures are not desirable.

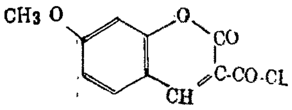
The separation of colourless compounds by chromatography may be facilitated in various ways. One of the methods may be to convert a colourless compound into a coloured derivative, which could then be separated by chromatographic adsorption. 2:4-dinitrophenylhydrazine has been used in the separation of carbonyl compounds, for instance, in the case of geronic acid and laevulinic acid (18). The yellow hydrazones could then be treated to regenerate the original components of the mixtures. Aromatic amines and hydrocarbons have been converted into their coloured picrates for this type of separation and advantage has been taken in the case of the phenols, of their coloured derivatives with ferric chloride. Azobenzene-*p* carboxylic chloride has been used in the separation of glucose and fructose by forming the coloured carboxylates and then developing the chromatogram (14).

The adsorption of colourless non-flourescent substances on a column of fluorescent material has been successfully carried out. Thus when a colourless compound is adsorbed on a column of a fluorescent material, which is then illuminated with ultra-violet light, it absorbs a part of the light and the zones of the column in which it is present fluoresce less strongly than the "empty" zones forming dark rings on a bright back-ground. H. Brockmann and F. Volpers (5) have developed this technique by making a proper selection of suitable fluorescent adsorbents. Zinc sulphide phosphors, which have been used by Sease (15), were found to be disappointing because of lack of adsorptive power, while the more common adsorbents used in chromatography showed very little or no fluorescence under the analytical lamp. The very faint bluish fluorescence of alumine can be sufficiently increased for the desired purpose by heating it to high temperatures with certain metals, but it is a tedious process when carried out on a large scale.

Therefore, Brockmann and Volpers (*loc. cit.*) have investigated the adsorption on alumina of fluorescent substances, which (*i*) would not react with the compounds and (*ii*) would not be removed during the process of elution. They have successfully used morin (magnificent yellow fluorescence), diphenylfluorindene sulphonic acid (red fluorescence) and salicylic acid (blue fluorescence). In day-light, the chromatogram zones are not perceptible but under ultra-violet light, they form dark rings on a bright background.

An essentially different approach gives great promise, namely, to produce a fluorescing compound by a clear-cut chemical reaction of two or more non-fluorescing organic compounds. The appearance of fluorescence then indicates that the reaction has taken place and that, consequently, certain compounds or groups must be present in the materials being tested. This has been suggested by F. Feigl (11).

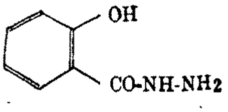
FLUORESCENT REAGENTS. Another general possibility is to combine the colourless compound with a fluorescent reagent, so that the derivative may be located on the column by its fluorescence in ultra-violet light (18,20). These procedures had not been investigated until in a recent paper, W. Baker and C. B. Collis (1) described some fluorescent acylating agents derived from 7-hydroxycoumarin for facilitating the separation and identification of hydroxy- and amino-compounds by chromatographic methods. The compound 7-methoxycoumarin-3-carboxylic chloride (I) has been prepared by these authors and is an excellent fluorescent reagent.



(I)

The range of these fluorescent reagents has been extended by W. Baker, C. N. Haksarand J. F. W. McOmie (2). A large number of papers have appeared in which the authors have tried to correlate fluorescence and chemical constitution, but it must be admitted that there are no rules that are generally applicable. In organic compounds, special fluorescent properties appear to be attendant upon given structures, the variation from which would alter or completely inhibit fluorescence. This constitutive property of fluorescence is often seen in the aromatic, cyclic or closed ring structures and bright fluorescence could be expecte[illegible]om polycyclic aromatic and heterocyclic compounds. Notable in[illegible]ances of such fluorescent structures are the molecules of salicylic acid and coumarin.

DERIVATIVES OF SALICYLIC ACID. Salicylic acid and certain of its derivatives are known to emit medium blue fluorescence. Therefore, several fluorescent reagents from this nucleus were prepared and studied by Baker, Haksar and McOmie (*loc. cit.*). The compounds salicylhydrazide (II) and *o*-methoxybenzhydrazide



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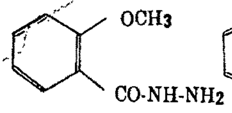

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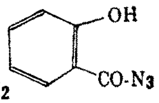
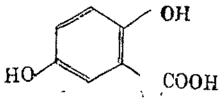


(IV)

(III) were used by these authors to characterise carbonyl compounds and equally fluorescent hydrazones were obtained. On account of the varying amount of fluorescence produced on a column when exposed to ultra-violet light, these hydrazones could be located on the column and hence a separation could be made. Similarly salicylazide (VI), althoug unsuitable, could be coupled with amino-acids to give fluorescent addenda.

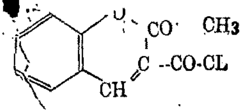
Broadly speaking, the groups hydroxyl, methoxyl, carboxyl, sulphonic acid and conjugated double bonds, all intensify the fluorescence, while others like halogens, nitroso and nitro, tend to quench it. As such, the molecule of 5-hydroxysalicylic acid (V) is more fluorescent than salicylic acid itself and derivatives from this are now being studied in this laboratory.



(V)

DERIVATIVES OF COUMARIN. The coumarin nucleus affords a highly flourescent system. T. R. Seshadri and coworkers (16) have discussed and summarized much of the available information concerning the constitutional factors that control visible fluorescence in the compounds of the benzo-pyrone group.

Baker, Haksar and McOmie (*loc. cit.*) have prepared and used coumarin-3-ca[illegible]xylic chloride (VI) and 7-methoxy-4-methyl coumarin-3-ca[illegible]xylic chloride (VII). These compounds are valuable fluorescent acylating agents and could be conveniently used for the characterisation of alcohols and amines. The possibility of using (VI) as a fluorescent reagent for phenols is now being explored by C. N. Haksar and S. K. Gupta (results



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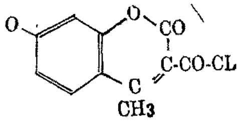

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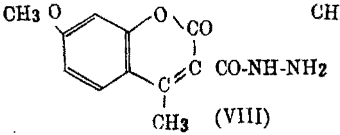

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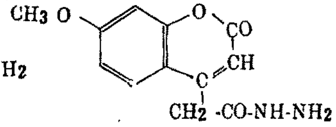
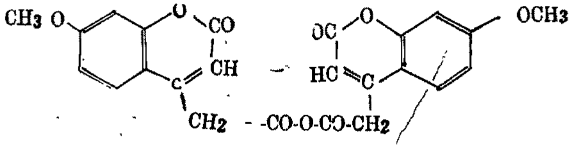


(IX)

not published yet). Some hydrazides, for example, 7-methoxy-4-methyldoumarin-3-carboxyhydrazide (VIII) and 7-methoxycoumarin 4-acethydrazide (IX) have been used as fluorescent reagents for carbonyl compounds (Baker *et. al.*, *loc cit.*). The hydrazide from coumarin-3-carboxylic however, acid, could not be obtained, due to the opening of thepyrone ring. Similarly, attempts to prepare 7-methoxycoumarin-4-acetyl chloride have failed. Another useful fluorescent acylating agent, namely, 7-methoxycoumarin-4-acetic anhydride (X) has now been prepared by the method used by F. H. Carpenter in the formation of the anhydride of benzyl penicillin (7).



(X)

This compound gives an intense blue fluorescence and is expected to give very good results when combined with hydroxy and amino-compounds.

REFERENCES

1. Baker W. & C.B. Collis. 1949. *J. chem. Soc.*, 12.
2. Baker, W., C.N. Haksar & J.F.W. McOmie. 1950. *J. Chem. Soc.*, p. 170.
3. Bowen, E.J. 1947. *Quart. Rev.*, **1** : 1.
4. Brewster, D. 1833, *Trans. R. Soc. Edinburgh*, 12.
5. Brockmann, H. & F. Volpers. 1947. *Berichte der Chemie*, **80** : 77.
6. Campbell, N. 1946. *Endeavour*, **5** : 155.
7. Carpenter, F.W. 1948. *J. American chem. Soc.*, **70** : 2964.
8. Cook, J.W. & al. 1933. *J. chem. Soc.*, 395.
9. Derberi, M. 1945. *Ann. chem. Anal.*, **27** : 31.
10. Dutt. S. 1930. *J. Indian chem. Soc.*, **7** : 505
11. Feigel, F. 1940. Specific and Special Reactions. New-York, p. 170.
12. Kuhn, R. 1931. *Z. physiol. chem.*, **197** : 141.
13. Nogrady, G. 1947. *Chem. Abst.*, **41** : 7573.
14. Reich, W.S. 1939. *J. Biochem.*, **33** : 1000.
15. Sease, J.W. 1947. *J. American chem. Soc.*, **69** : 2242.
16. Seshadri, T.R. & al. 1940. *Proc. Indian Acad. Sci.*, **12** : (A) : 375 ; 1941. **13** (A) : 316; 1942. **16** (A) : 68.
17. Strain, H. 1935. *J. American chem. Soc.*, 57 : 758.
18. Strain, H. 1941. Chromatographic Adsorption Analysis. New-York. p. 176.
19. Well & Williams. 1951. *Nature*, **167** : 906.
20. Williams, T. I. 1946. An Introduction to Chromatography, p. 50.

ACTION OF DRUGS AND IONS ON UNSTRIATED MUSCLE

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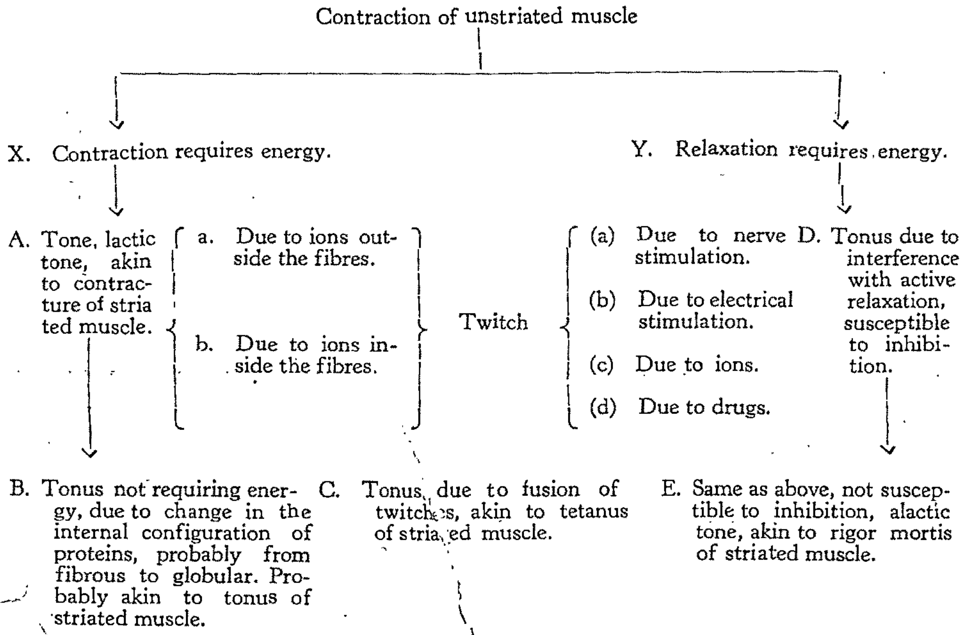
Unstriated muscle is a favourite object for study of the action of drugs and ions. Both excitation and inhibition may be studied and quantitative results obtained. Unstriated muscle is however a complex structure and unless its variables are kept in mind, the results are likely to be conflicting. The two chief variables in unstriated muscle are : (*i*) the excitatory mechanism (*ii*) the contractile mechanism. It has hitherto been assumed in studies of drug action that the contractile mechanism does not vary in its properties. This may be correct in most experiments, but the contractile system is likely to vary in its properties, its most important variation being its inability to relax.

Unstriated muscle shows two kinds of tone : one which is susceptible to asphyxia (*lactic tone*) and the other resistant (*alactic tone*). When the muscle is in alactic tone, the contractile mechanism is unable to relax, with the result that inhibitory substances such as adrenaline would not be able to produce relaxation. The excitatory system appears to be normal, as contraction by excitatory substances can be produced.

The responses of unstriated muscle are classified as follows (Singh and Singh, 1951 ; Singh, 1952.)


Contraction of unstriated muscle
X. Contraction requires energy.
Y. Relaxation requires energy.
A. Tone, lactic tone, akin to contracture of striated muscle.
a. Due to ions outside the fibres.
b. Due to ions inside the fibres.
Twitch
(a) Due to nerve stimulation.
(b) Due to electrical stimulation.
(c) Due to ions.
(d) Due to drugs.
D. Tonus due to interference with active relaxation, susceptible to inhibition.
B. Tonus not requiring energy, due to change in the internal configuration of proteins, probably from fibrous to globular. Probably akin to tonus of striated muscle.
C. Tonus, due to fusion of twitches, akin to tetanus of striated muscle.
E. Same as above, not susceptible to inhibition, alactic tone, akin to rigor mortis of striated muscle.

There are six ways (Aa,Ab,B,C,D,E,) in which unstriated muscle can produce sustained tension. Tension beginning as C, may pass into A, and then to B as in muscle of *Mytilus*, or to D, and E, as in mammalian and frog unstriated muscle. Tonus in mammalian and frog unstriated muscles consists of a variable mixture of A and E. Thus in the stomach muscle of dog and frog, the pyloric portion mainly shows alactic tone ; while the cardiac end exhibits lactic tone. Various smooth muscles, besides differing from one another in having variable mixtures of lactic and alactic tones, also differ from each other in their properties of having different capacities for exhibiting lactic tone and twitch contraction. There is no antagonism between alactic tone and twitch contraction, probably because they are subserved by different contractile mechanisms. Lactic tone and twitch are antagonistic, as they have to compete for the same contractile mechanism.

Inhibition in unstriated muscle is also of two kinds, one active and the other passive. Active inhibition can be abolished by poisoning the muscle with cyanide, when only passive inhibition remains. Besides, inhibition is also due to ions inside and outside the fibres, the two inhibitions being antagonistic (Singh, 1942).

The entrance of sodium into nerve during excitation is an established fact. Hodgkin and Katz (1949) have stressed its importance in the reversal of action potential. The entrance of ions from the saline into the muscle fibres appears to play a very important part in determining the type of response by unstriated muscle and drug reversals (Singh, 1944). The evidence for this is both chemical and physiological. Chemical analyses have shown that the unstriated muscle of *Mytilus* gains sodium during stimulation (Singh, 1939). The same muscle gains ions from the saline in the absence of calcium (Singh, 1938, 1939, 1944b). The gain of ions by the muscle is increased by blotting.

Physiological evidence for the entrance of ions into the muscle fibres during stimulation is as follows. If *Mytilus* muscle is immersed in saline containing barium ions, it passes into a tonic contraction. At other times, especially in summer, the muscle may remain quiescent without any apparent effect. Barium ions are present in the saline in both instances, and yet the muscle remains quiescent in one instance and contracts in the other. The quiescent muscle can be made to produce a typical barium contraction by certain methods, such as passage of electric current for a short period insufficient to cause a contraction or produce only a twitch, sudden heating or cooling, sudden stretch or release, stroking the surface with a hair brush or blotting paper, or vigorous aeration with air or an indifferent gas such as nitrogen, removal of calcium and addition of drugs like adrenaline, acetylcholine, caffeine. These drugs, by themselves, may or may not produce a contraction.

The above factors appear to be unrelated, but there must be some common effect produced by them. The removal of calcium suggests increase in permeability and entrance of barium into the muscle. As mentioned above, this is supported by chemical analyses. Blotting also, as shown by chemical analyses, increases the gain of ions by the muscle. Two conclusions follow from the above experiments : (a) The common factor among the above agencies is that they produce excitation, and during such excitation, ions enter the muscle fibres from the saline. (b) To produce excitation ions must enter the muscle fibres. This latter conclusion is supported by chemical and physiological experiments. The stimulating power of many ions is proportional to their ability to enter the muscle (Singh, 1938, 1939). The above argument regarding barium, applies to other ions in the saline, such as sodium and potassium. The cessation of excitation would be followed by extrusion of these ions by the muscle fibres.

To produce excitation, therefore, an ion has to enter the muscle fibres. During such excitation, however, other ions also enter and modify the response produced by the original substance. If the original substance is excitatory, then excitatory ions in the saline would enhance its action and inhibitory ions would have the opposite effect, the latter ions, may, in fact, reverse the action of the original substance. Similarly the inhibitory action of a substance may be decreased or reversed by an excitatory substance. The entrance of ions from the saline into the muscle fibres is responsible for excitation persisting after the cessation of the stimulus, producing after discharge or slow relaxation.

It appears that not only excitatory but also inhibitory ions may enter the muscle fibres and produce inhibition. Thus the action of sodium chloride in frog muscle is inhibitory and removal of calcium produces relaxation instead of contraction. Similarly, adrenaline may produce excitation, if there is excess of excitatory ions in the saline and inhibition if there is excess of inhibitory ions, such as hydrogen ions. This appears to be one cause of drug reversal.

REFERENCES

Hodgkin, A.L., and Katz, B. 1949, *J. Physiol.*, **108** : 37.
Singh, I. 1938. *ibid.*, **91** : 398.
Singh, I. 1939 *ibid.*, **96** : 1.
Singh, I. 1942. *Indian J. med. Res.*, **30** : 629.
Singh, I. 1944 a. *Proc. Indian Acad. Sci.*, **20** : 195.
Singh, I. 1944 b. *ibid.*, **20** : 208.
Singh, I. 1952. *Curr. Sci.*, **21** : 77.
Singh, S.I. and Singh, I. 1951. *Proc. Indian Acad. Sci.*, **33** : 165.

A CLINICAL STUDY OF THREE HUNDRED AND EIGHTY-THREE CASES OF TYPHOID FEVER AT THE S. N. HOSPITAL, AGRA

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This paper summarizes the results of studies on the incidence, symptomatology, complications, etc., of typhoid fever, and is based on the clinical records of the typhoid patients admitted in the S. N. Hospital, Agra, from January 1941 to November 1950. These cases include almost all the clinical manifestations encountered by practising physicians.

Incidence

The incidence of typhoid fever has been slowly rising from 1941 to 1948, except in 1944 and 1947, when the yearly incidence showed a slight decrease. In 1948 the incidence was highest and then has shown a gradual decline.

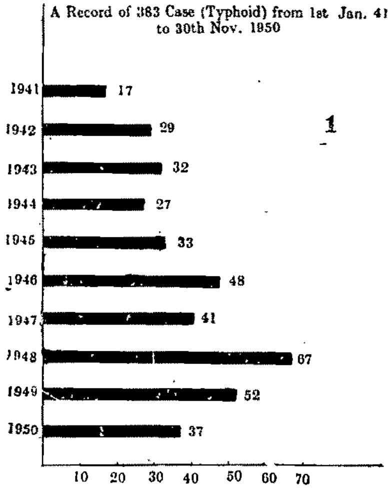
Although typhoid cases were seen throughout the year in all the seasons, the maximum incidence occurs in summer and early rainy season. The incidence starts increasing from March till it reaches its maximum in August and September, after which it shows a sharp decline, coming to a minimum in the months of December and January (see graph in Fig 2). This is due to the increase in the fly population, which is the most important and significant factor in the spread of the disease.

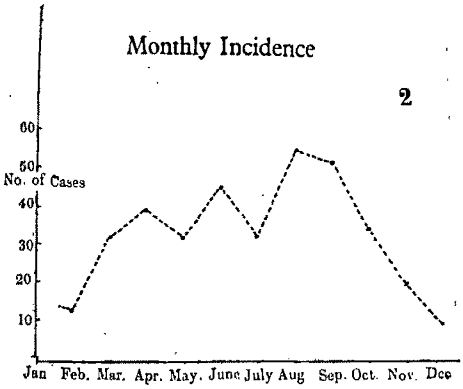
Racial Distribution

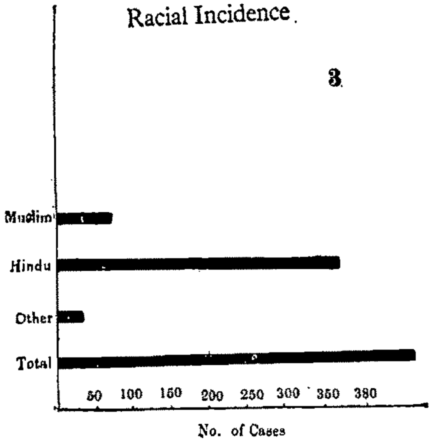
Of the 383 cases investigated, 300 were Hindus, 60 Muslims and 23 others (Fig. 3). This is not however proportional to the Hindu and Muslim population of this part of the country. The cause of the increased susceptibility of the Hindu community cannot at present be accounted for in any way.

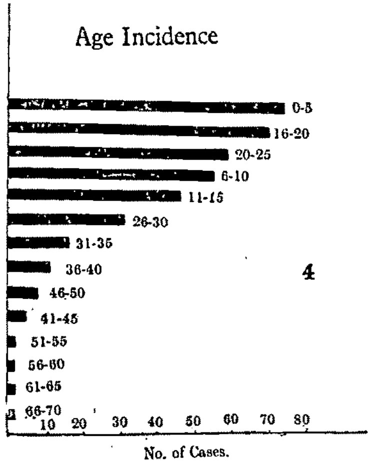
AGE INCIDENCE

As we see in the accompanying block graph (Fig 4.), no age exempt, though it is extremely uncommon after 50 years of age. In the present study the largest number of cases was encountered in the age group of 0-5 years. The incidence then gradually falls upto 15 years of age, after which there is a sudden rise, followed by a gradual decline. The oldest patient in this series was a man of 70 years. Out of 383 cases, 129 were children.









SEX INCIDENCE

In the present series 314 patients were males and 69 females. This shows that 82% of these cases were males and the tres famales. This can be accounted for by the fact that because of their occupation, males are much more liable to catch the infection than the females, who lead a relatively sheltered life.

SOURCE OF INFECTION

The exact source of infection could not be traced in these cases, because of complications, of the irregular dietetic habits, insanitary conditions of living, etc. In comparatively few cases alone was it possible to trace the source to the disease occurring in their families. In the majority of such cases the source of water supply was from the municipal pipes, in only a few from wells, but none from tanks. In the majority of the cases, from the various points in the history, the infection could only be attributed to the insanitary conditions of the locality.

INCUBATION PERIOD

From the date available in the histories, calculation of the incubation periods is rather difficult. History of the exposure to infection is available in a very few cases only. From a study of the onset of the disease from the latter group i.e., where history of exposure to infection has been available, it can be taken to vary from 8 to 21 days. This is probably proportional to the number of bacilli infecting the individual. Average duration of disease before admission has been 17.9 days, minimum duration of disease before admission 1 day, and maximum duration of disease before admission 150 days.

ONSET

Out of 383 patients 11 cases gave the history of sudden onset and in the rest there was an insidious onset.

PRODROMAL SYMPTOMS

As shown in the accompanying table, the respiratory symptoms in the form of slight cough was the commonest prodromal symptom present in 63 cases (16.4%). Headache was observed in 41 cases (10.9%), and anorexia in 35 cases (9.4%), sore throat in 27 cases (7%) and abdominal pain in 26 cases (6.8%).

A small percent of cases (10.2%) gave a history of chill at the onset of the disease, before any prodromal symptoms were noticed. None however complained of chilliness or shivering during the course of the disease.

Other less important prodromal symptoms were malaise, diarrhoea and constipation. Most of the patients complained of two or more than two prodromal symptoms.

Prodromal symptoms

Symptoms	No. of cases	Percentage
Malaise ...	14	3·68
Headache ...	41	10·99
Anorexia ...	35	9·39
Cough ...	63	16·44
Sore Throat ...	27	7·08
Early abdominal pain ...	26	6·79
Early diarrhoea ...	16	4·2
Early constipation ...	18	4·69

TEMPERATURE

After the actual onset of the disease with slight chilliness or otherwise, the temperature records showed many variations. In most of the cases no temperature records were available till they were admitted in the hospital. On admission and on the subsequent days different temperature charts conformed to the three types (i) the remittent, (ii) continuous and (iii) the intermittent. In 257 out of 383 cases (67.1%), the fever was remittent and the daily variation in the temperature was 1.5°F. or more. In 77 cases (20.1%) the fever was of the continuous type, with a daily variation usually ranging between 1 to 1.5° F. In a small percentage of cases (12.8%) the fever was of the intermittent type.

Type of fever

Type	No. of cases	Percentage
Continuous ...	77	20·10
Remittent ...	257	67·10
Intermittent ...	49	12·80
Chill at onset ...	39	10·18

The minimum duration of the fever in the present study was 7 days and a maximum was 154 days, with an average of 27.3 days.

In most of the cases the fever showed the three characteristic stages i.e., the "step-ladder" rise, the festigium and the defervescence, but a few also showed a sudden rise of temperature, reaching its peak not in the usual six to ten days period of the "step-ladder" stage. In some cases hyperpyrexia was also observed.

Relapses were also seen in some cases. These were akin to the initial attack in some, but in most of these the duration of the relapse period was a short one.

GASTRO-INTESTINAL MANIFESTATIONS

The essential lesion in the typhoid fever being in the lymphoid follicles of the small intestine, the gastro-intestinal manifestations are multifarious.

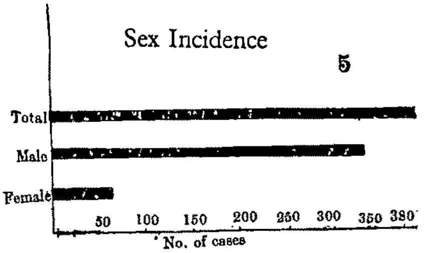
Gastro-intestinal manifestations

Symptoms	No. of cases	Percentage
Diarrhoea ...	40	10·4
Abdominal distension ...	57	14·48
Constipation ...	49	12·8
Abdominal tenderness ...	43	11·3
Abdominal pain ...	26	6·8
Bleeding from gums ...	6	1·6
Vomiting ...	5	1·3
Haemorrhage ...	12	3·13
Parotitis ...	1	0·26
Splenic enlargement ...	66	17·23
Jaundice ...	2	0·52
Hepatic enlargement ...	28	7·31
Hiccough ...	1	0·26
Palpable gall bladder ...	1	0·26
Perforation and peritonitis ...	2	0·52
Sordes ...	2	0·52
Dysphagia ...	1	0·26

Anorexia is the commonest complaint of this disease and this was observed in almost all of the cases from the very beginning of the disease. In most of the cases this had been present for a few days before the onset of the fever. As anorexia decreased, the appetite returned, with the improvement in the patient's condition, so much so that during the convalescent stage some patients cried for food.

Diarrhoea was seen as a prodromal symptom in 16 cases (4.2%), but during the actual course of the illness the incidence increased to 40 cases (10.4%). These cases include mild and severe diarrhoeas. Most of the cases who got diarrhoea suffered from greater complications in the disease than those who were constipated.

Sex Incidence



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Average duration of disease before admission 17·913 days.

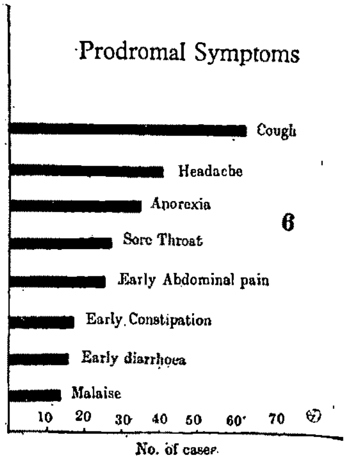
Minimum „ „ „ „ „ 1 day.

Maximum „ „ „ „ 150 days.

Average duration of stay in the hospital 39·47 days.

Maximum „ „ „ „ „ „ 122 days.

Prodromal Symptoms



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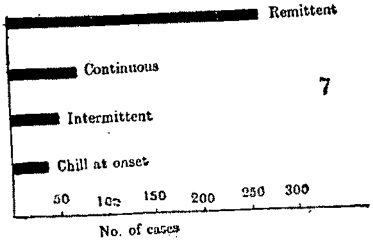
Type of Fever



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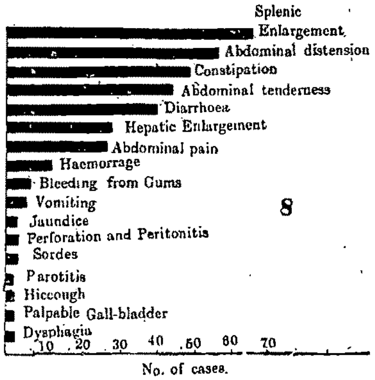
N.B. The temperature record indicates fever during hospitalisation only.

Gastro-Intestinal Manifestation

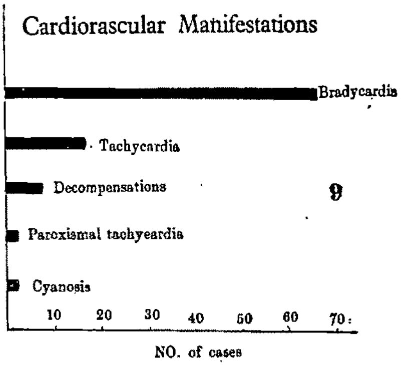


8

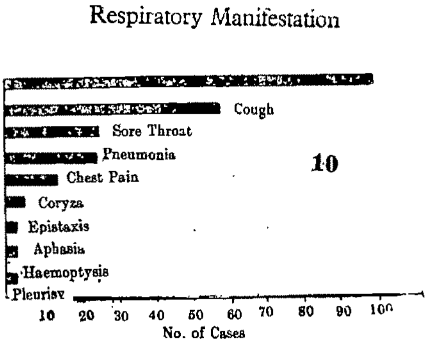
Constipation was more frequent than diarrhoea and was observed in 49 cases (12.8%). This however does not include those cases in which constipation developed after the diarrhoea had ceased. In order to overcome the constipation, enemas were given on alternate days and in one case the constipation became so obstinate that the enema failed to produce the desired result, and a regular digiial removal of the faecoliths had to be carried out. In none of the cases however complications had developed.

Abdominal tenderness was seen in 43 cases (11.25%), while abdominal pain, as distinct from tenderness, was seen in 26 cases (6.79%). These manifestations were common after the end of the first week of the illness and although present all over the abdomen, was specially more marked in the right iliac fossa.

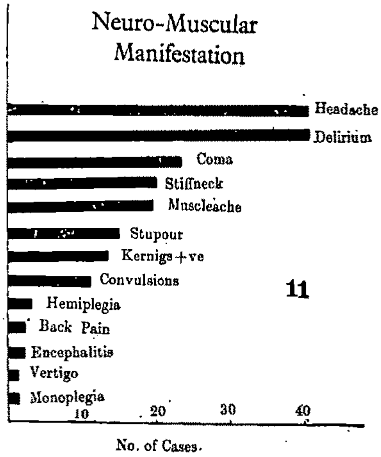
Abdominal distension was observed in 57 cases (14.48%) of the 383 cases. The distension was more marked in the second week, but gradually subsided as the fever came down and toxoemia decreased. It was in these cases of abdominal distensions that intestinal haemorrhage and perforation occurred. Intestinal haemorrhage occurred in 12 cases (3.13%) and perforation and peritonitis developed in 2 cases (0.52%).


Cardiorascular Manifestations

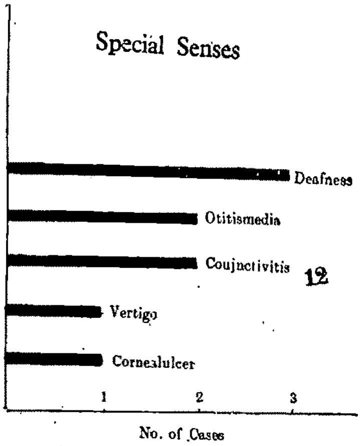
9


Respiratory Manifestation

10


Neuro-Muscular Manifestation

11


Special Senses

12

Splenic enlargement was detected in 66 cases (17.23%). The enlargement was slight and the spleen was felt as a soft mass below the left subcostal margin. The splenic enlargement disappeared with the clinical improvement. Other gastro-intestinal manifestations included bleeding from gums, dysphagia, sordes vomiting, parotitis, etc.

LIVER AND GALL-BLADDER MANIFESTATIONS

Clinical evidence of liver and gall-bladder manifestations was present in 7.31% of the 383 cases. In some of them, it was a manifestation of congestive cardiac failure and in some others the enlargement could be attributed to the typhoid infection. In the latter group the hepatic enlargement decreased with the clinical improvement of the case. Clinical evidence of jaundice was seen in 2 cases (0.52%) only. The hepatic function was, however, not determined by the liver function-test study. Typhoid hepatitis was observed in 2 cases (0·52%) of the present study. The incidence of the typhoid hepatitis is very rare indeed. Clinical evidence of cholecystitis was found in 1 case only (I.26%). An attempt to isolate the carriers after the stool and bile culture was not done.

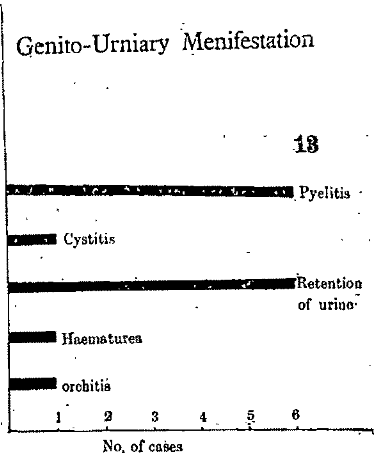
CARDIOVASCULAR MANIFESTATIONS

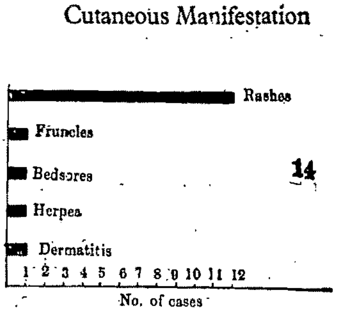
The symptoms relating to the cardiovascular system were not much in the present study. These symptoms were chiefly recorded during the earlier period of the disease and the commonest manifestation was bradycardia. In one case bradycardia appeared on the first day of the fever and this led to the possibility of the typhoid attack on the very first day of pyrexia. Along with bradycardia dicrotism was also noticed in a few of these cases. Tachycardia was observed in the early stage in about 4·7% of the cases. Paroxysmal tachycardia was observed in 1 case. The cases which showed tachycardia in the beginning were usually serious.

Blood pressure readings in the early stages of the disease did not show any abnormality, but as the disease progressed, the blood pressure declined. Signs of venous thrombosis were not seen in any of the cases.

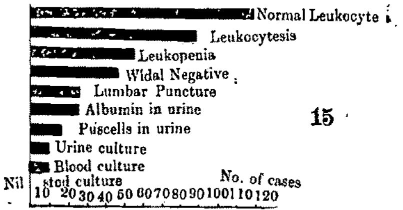
RESPIRATORY MANIFESTATIONS

Evidence of respiratory tract involvement in some form was present in almost all the cases. The commonest manifestations were bronchitis, cough, sore throat and bronchopneumonia. Coriza was present only in 5 cases. Bronchopneumonia was met with in several cases, due to a secondary infection. The respiratory rate was not increased in these cases, except when complicated by pneumonia or intestinal haemorrhage or perforation. Chest pain was seen in 14 cases, an incidence of 3.7%. Pleurisy was seen in none of the cases. Epitaxis was seen in 2 cases only. One case had haemoptysis and in this case there were signs of bronchitis and intermittent temperature, so pulmonary tuberculosis was suspected, but acid fast bacilli were not

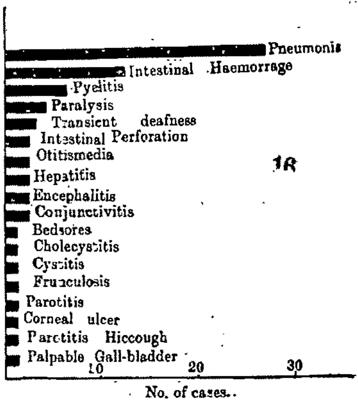




Average Duration of fever 27·29 days
Minimum „ „ „ 7 days
Maximum „ „ „ 154 days



Bacillus Typhosum, H positive in 160 cases (Lowest titre being 1/25 & highest being 1/2500)
Bacillus Paratyphoid A, H positive in 48 cases.
„ „ B, „ „ 30 cases.
Total O was done in 22 cases.
Viantigen, Positive in 4 cases.
O & H both Positive in 10 cases.
O alone was Positive in 7 cases.
H „ „ „ „ 165 cases.
TAB were Positive with O in two cases only.
Widal Typhoid 130 cases 33·94%.
„ Para A 24 „ 5·99%.
„ Para B 23 „ 5·74%.



found. Skiagram of the chest did not also reveal any abnormality in the lungs. Cyanosis was seen in one case only.

Respiratory manifestations

Manifestations		No. of cases	Percentage
Coryza	...	5	1·30
Cough	...	59	15·11
Sore throat	...	27	7·05
Bronchitis	...	100	26·11
Epitaxis	...	2	0·52
Aphasia	...	2	0·52
Chest pain	...	14	3·7
Pneumonia	...	27	7·05
Haemoptysis	...	1	0·26
Pleurisy	...	...	...

NEUROMUSCULAR MANIFESTATIONS

The outstanding symptoms noted were delirium, (10.4%) and headache (10.4%). The delirium was of a low muttering type in most of the cases, but some of the patients showed violent tendencies. None of them however showed psychosis, meloncholia or mania. The other outstanding manifestations were coma, (5.7%), stiff neck (5.2%) and a positive Kernigh's sign (3.4%), muscle ache (5%) and stupor (4%). Typhoid encephalitis and meningism were observed in 15 cases to warrant lumbar puncture to relieve the increased intra-cranial tension.

Neuromuscular manifestations

Symptoms		No. of cases	Percentage
Headache	...	40	10·44
Muscle ache	...	19	5
Convulsions	...	11	2·87
Backache	...	2	0·52
Delirium	...	40	10·44
Stupor	...	15	4·1
Coma	...	23	5·7
Stiff Neck	...	20	5·2
Kernig's positive	...	13	3·4
Vertigo	...	1	0·26
Hemiplegia	...	3	0·78
Monoplegia	...	1	0·26
Encephalitis	...	2	0·52

Backache, although reported in the literature to be quite common in such cases, was rather a rare manifestation in this series. Paralytic lesions in the form of hemiplegia (3 cases) and monoplegia (1 case) were also observed, as a result of which convalescence was quite prolonged. Flexion contractures were, however, not observed. Aphasia was seen in two cases.

SPECIAL SENSES

Severe aching of the eye muscles and photophobia were never met with in these cases. Conjunctivitis was seen in 2 cases and corneal ulcer in one.

Transient partial deafness was met with in 3 cases and vertigo in one. Otitis media occurred in 2 cases but mastoiditis was never found in any case.

Special senses

	No. of cases	Percentage
Otitis media ...	2	0·52
Deafness ...	3	0·78
Vertigo ...	1	0·26
Conjunctivitis ...	2	0·52
Corenal ulcer ...	1	0·26

GENITO-URINARY MANIFESTATIONS

The genito-urinary manifestations in the study are few and yet interesting. Pyelitis was seen in 6 cases out of 383 and cystitis in only one.

Retention of urine occurred in 6 cases. One developed haematuria and 1 orchitis, a very rare manifestation. An attempt to establish bacilluria was not made in this study.

Genito-urinary manifestations

	No. of cases	Percentage
Pyelitis ...	6	1·56
Cystitis ...	1	0·26
Retention of urine ...	6	1·56
Haematuria ...	1	0·26
Orchitis ...	1	0·26

CUTANEOUS MANIFESTATIONS

The commonest skin manifestation met with in this series is the typhoid rash, the rose spots which were seen in 12 cases (3.13%). Although a large percentage of patients have been reported to develop this rash, in this study the figure of incidence is rather low. This is probably due to the dark colour of the skin, where rash does not become manifest. Whenever a rash was seen, it was on the trunk. The intensity of the rash was very mild in all the cases and in practically all of them the rose spots were very few.

Cutaneous manifestations

Types	No. of cases	Percentage
Rashes ...	12	3·13
Furuncles ...	1	0·26
Bed sores ...	1	0·26
Herpes ...	1	0·26
Dermatitis ...	1	0·26

Bed sores, furuncles and dermatitis were observed in one case each. The small percentage of bed sores is perhaps due to the proper nursing and the maintenance of the cleanliness of the patients. In the case of furuncles, typhoid bacilli were not isolated from the discharge. The case which developed herpes was not complicated by pneumonia.

LABORATORY DATA

2. *Blood culture.* —This was done as a routine in those which happened to come early in the first week or so of the disease, and in a few cases the blood cultures were repeated more than twice. Positive blood cultures were seen in 9 cases, an incidence of 2.4%. Although it is not possible to account for these low values, the fact that the cultures was probably done too late may have something to do. The incidence of positive blood culture has been very low, as compared with positive Widal reaction in the same case (Widal reaction of course having been done much later on in the course of the disease, which will be discussed shortly). On account of this difference, the blood cultures should not be delayed as far as possible.

Laboratory investigations

	No. of cases	Percentage
Leucopenia ...	54	20·85
Leucocytocis ...	88	33·97
Normal leucocytocis ...	177	45·18
Pus cells in urine ...	17	4·43
Albumin in urine ...	25	6·25
Widal negative ...	47	17·34
Urine culture+ ...	9	2·24
Blood culture+ ...	9	2·24
Stool culture+ ...	0	0·00
Lumbar puncture ...	27	7·21

2. *Widal test.*—This test was carried out in 271 cases and if it was found to be negative, it was repeated. Even in the re-examination of this test, it was found to be negative in 17·34% of the cases.

Widal test positive against H agglutinins in high titres was considered to be diagnostic of typhoid fever. It was positive against *B. typhosus* in 160 cases, against Para A in 48 cases and against Para B in 30 cases.

Bacillus typhosus, H positive in 160 cases (lower titre being 1/50 and highest being 1/2500)
Bacillus paratyphoid A, H positive in 48 cases.
" " B, " " " 30 cases.
Total O was done in 22 cases and was positive in 17 cases.
Vi Antigen, positive in 4 cases.
O & H both positive in 10. cases
O alone was positive in 7 cases.
H " " " " 165 cases.
T A B were positive with O in 2 cases only.

Agglutination against O agglutinins was done in 22 cases and was found to be positive in 17 cases. Out of these 17 cases 10 had Widal positive against both H and O agglutinins, whereas in 7 cases agglutination was only positive against O and completely negative against H. Widal was positive against B typhosus, Para A and Para B, i.e., all the three, in 2 cases only. Vi-antigen was positive in 4 cases only.

3. *Stool culture.*—This was not done in this series.

4. *Urine examination.*—Albuminuria was seen in 25 cases (6.25%), mostly a febrile reaction. Pyuria was seen in 17 cases and positive urine cultures were obtained in 9 cases only.

5. *Blood picture.*—This was done in 259 cases and in most of these the investigation was repeated more than once. In 111 cases (45.18%) out of the 259, the total and differential leucocyte counts showed a normal figure, 54 cases (20.85%) showed leucopenia and 88 cases (33.97%) showed leucocytosis. Blood film was examined in all the cases for M. P. and it was negative.

COMPLICATIONS

The commest complication was pneumonia, intestinal haemorrhage, pyelitis, etc.

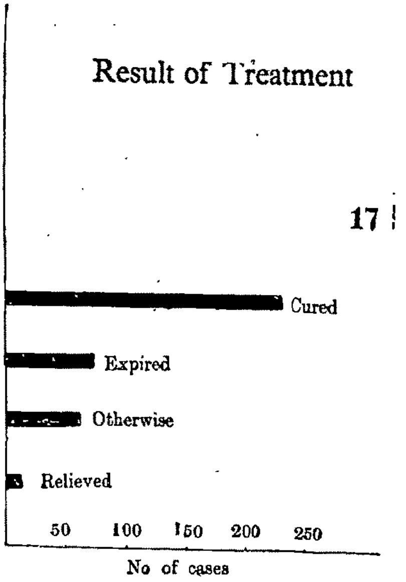
Complications

Complication	No. of cases	Percentage
Intestinal haemorrhage ...	12	4·13
Bed sores ..	1	0·26
Pneumonia ...	27	7·05
Cholecystitis ...	1	0·26
Cystitis ...	1	0·26
Furunculosis ...	1	0·26
Intestinal perforation	2	0·52
Pyelitis ...	6	1·56
Otitis media ...	2	0·52
Transient deafness ...	3	0·78
Parotitis ...	1	0·26
Hepatitis ...	2	0·52
Encephalitis ...	2	0·52
Corneal ulcer ...	1	0·26
Coujunctivitis ...	2	0·52
Paralysis ...	4	1·04
Periostitis ...	1	0·26
Arthritis ...	1	0·26
Orchitis ...	1	0·26

DIAGNOSIS

An indisputable diagnosis, of course, can only be made after the typhoid bacilli have been cultured from the blood, stool or urine. In endemic areas and in epidemics, the diagnosis is mostly clinical, but in a full-fledged case of typhoid inadequate historical data are usually forthcoming and as such all diagnostic procedures must be carried out in all the cases.

A tentative diagnosis can be made from the prodromal symptoms—insidious onset, "step-ladder" rise of temperature, a slow pulse, dicrotism, headache, bronchitis, typhoid rash, leucopenia, anaemia and splenic enlargement and the characteristic fall of the temperature by lysis.



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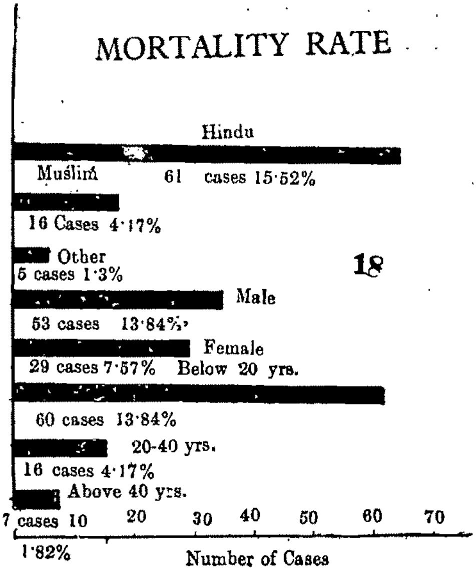

18

The laboratory data, if positive, stamp the diagnosis of the disease, but a negative reaction does not rule out the condition, because in a fairly good number of cases the laboratory investigations have been of practically no help.

If the typhoid bacilli are demonstrated in the blood by culture, the diagnosis is certain. Widal test, although a reliable test after the tenth day of the fever, has certain fallacies. Occasionally it comes out positive quite late in the disease (in one case it was positive on the 29th day, the earlier tests having been entirely negative), or it is positive in very low titres and hence the diagnosis could not be made on this ground alone, and so the importance of repeated Widal test, when a positive reaction in a rising titre is diagnostic. Possibility of anamenestic reaction should always be borne in mind and this can be ruled out by doing agglutination tests against O agglutin in all the cases.

COURSE OF THE DISEASE

The average duration of the disease before admission to the hospital was 17.9 days, but the time of admission varied from 1 day to 150 days. The average duration of stay in the hospital was 39.47 days, whereas the maximum was 120 days.

RESULT OF TREATMENT AND MORTALITY RATE

These are shown in Figs. 17 & 18.

Results of treatment

	No. of cases	Percentage
Cured ...	233	60·08
Relieved ...	12	3·13
Other course ...	60	15·6
Expired ...	78	20·37

Mortality rate

Details	No. of cases	Percentage
Hindus ...	61	15·52
Muslims ...	16	4·17
Others ...	5	1·30
Male ...	53	13·84
Female ...	29	7·57
Below 20 years ...	60	15·6
20-40 years ...	16	4·17
Above 40 years ...	7	1·82

ACKNOWLEDGMENTS

I am thankful to Dr. H.N. Bhatt, M.B., F.R.C.S.E., D.M.R.E., Principal & Superintendent, Medical College, Agra for his kind permission to utilize the records of cases of the S. N. Hospital, Agra.

STUDIES OF THE ROLE OF FUNGI IN PULMONARY DISEASE*

By P. N. WAHI, M. D., M. R. C. P. (LOND.), *Department of Pathology, Medical College, Agra.*

In the recent years it has been realized that there is a disease or a group of diseases which closely simulate tuberculosis. This has been indicated by the observation of pulmonary calcification in negative reactors to tuberculin. Until the past decade this was attributed either to possible reversal or loss of tuberculin sensitivity or suppression of the same due to certain infections, extreme debility or overwhelming tuberculous infection. In the light of last few years' investigations the more probable explanation is the occurrence of a second disease, which may cause intrathoracic calcification. The more recent advances in the knowledge of coccidioidomycosis and histoplasmosis in this connection are noteworthy and are to a large extent attributable to circumstances arising out of second world war.

Till recently coccidioidomycosis and histoplasmosis were considered as rare diseases confined to small endemic areas in the United States—the former in the south-western part and the latter in the central eastern regions. During the past decade these diseases, especially histoplasmosis, have been found widespread in the United States and in widely scattered places throughout the world.

HISTORICAL REVIEW

In the United States the number of tuberculin negative individuals with pulmonary calcification was too great to be explained by loss of tuberculin sensitivity. It was then thought that agents other than tubercle bacilli might be responsible for intrathoracic calcification. In 1938 Dickson and Gifford[16] wrote that the benign infection called valley fever, characterized by

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*Supported by a grant from Indian Council of Medical Research.

pneumonia and erythema nodosum represented initial benign stage of coccidioidomycosis and the granuloma or dessiminated form was a more serious late stage of systemic disease. This concept was further supported by Cox and Smith[10] and Aronson *et al.*[2] by the demonstration of role of the fungus, *Coccidioides immitis* in the production of pulmonary disease in certain localised regions of California. With the establishment of pathogenesis of the disease, further advance in epidemiology and diagnosis was achieved by the application of skin sensitivity tests, serologic, complement fixation, and precipitin tests. It was found that upward of 25 per cent of army personnel stationed in the endemic areas were infected.

Important contribution to the clinical manifestations of coccidioidomycosis was made by Bass[3] and Bass *et al.*[4,5] They found the residual lesions of different types, *i.e.*, nodular densities, cavities, mottled infiltrations, fibrosis, pleural effusion and calcification. The lesions resembled tuberculosis. All the patients reacted to coccidioidin in 1 : 100 dilution. However, an occasional case reacted positively to both tuberculin and coccidioidin tests.

In 1943 Smith[40] found positive coccidioidin reaction in south-western Ohio and pointed out that this area was not in the endemic region of coccidioidomycosis. He pointed out that this was the area of pulmonary calcification in tuberculin negative persons and also the endemic area of histoplasmosis. He therefore in 1943 first suggested *Histoplasma capsulatum* as a possible specific agent. It was also at this time that cross reaction between different fungi was considered an important possibility [18,40] and this has since then been further substantiated.[24] Recently Smith *et al.*[10] have published the observations of Furcolow and Nelson that as long ago as 1940, a fungus other than *Coccidioides immitis* was considered a possible cause of intrathoracic calcification in tuberculin negative children in an orphanage in Ohio.

This suggestion of Smith[40] was taken up by other workers especially by Christi and Peterson[7,8,9,37] in their important studies of pulmonary calcification in Tennessee. They demonstarted an association between histoplasmin sensitivity and pulmonary calcification in tuberculin negative reactors. In brief, their results indicated that the number of children positive to histoplasmin and showing pulmonary calcification was more than three times those of pulmonary calcification with positive sensitivity to tuberculin. They found that 43·6 per cent of children revealed intrathoracic calcification on roentgen examination. Of these 10 per cent were negative to both tuberculin and histoplasmin. The number of persons with positive reactions and with pulmonary calcification increased with age. The curves of pulmonary calcification and histoplasmin sensitivity followed closely while the curve of tuberculin reactors divulged significantly from those of pulmonary calcification and for histoplasmin sensitivity. They also found an appreciable decrease in the incidence of calcification in the second decade.

Palmer[34] in 1945 tested 10,000 nurses in 65 schools scattered throughout the United States and found that the highest prevalence of histoplasmin sensitivity was in the central States. This was in accordance with the findings of other workers.[27,42] This original geographical location of the disease has since then been confirmed by studies of school children,[7, 8, 9, 19, 20, 24, 25] of university students[6, 38] and of nurses.[23, 34, 35, 38]

Prior and Allen[38] found the incidence of positive histoplasmin reactors among rural residents significantly greater than among urban residents. This finding is just the opposite of tuberculin reactors. Ferebee and Furcolow[19] reported greater incidence of positive reactions in siblings, if the oldest child was positive, than otherwise. They further found a similarity in reaction between siblings, but as the children became older the similarity diminished. They came to conclusion that geographic factors were more important than racial factors in producing sensitivity. They also suggested that there were only slight differences in sensitivity to histoplasmin by sex.

The most important finding has been the constant large percentage of positive skin histoplasmin tests in individuals with intrathoracic calcification.[1, 6, 11, 13, 17, 19, 20, 23, 25, 35, 38, 47, 48]

Furcolow *et al.* have reported that out of 828 children with pulmonary calcification, 78·4 per cent reacted positively to histoplasmin. This figure went up to 93·5 per cent when children with disseminated calcification were considered. Similar has been the findings of other workers.[7, 8, 9, 23, 25, 44, 49] The highest figures were reported from areas of endemic histoplasmosis. Even in non-endemic areas a close parallelism has been reported between the two. Absher and Cline[1] observed the same in their studies in Rockey Mountain area. It has, however, to be reckoned that the incidence of positive reactors diminishes as one goes farther away from endemic areas.[38]

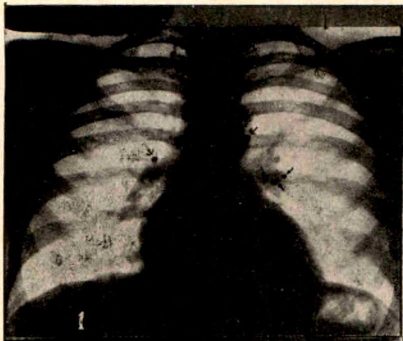
Blastomycosis is another fungus infection in the lungs which can be misdiagnosed as tuberculosis, until dissemination of the infection with the formation of subcutaneous abscesses occurs. Primary infection in the lungs does not show any characteristic roentgenographic findings[46]. A number of epidermal tests for blastomycosis have been tried.[13, 26, 30 33, 45] Martin and Smith[29] reported that at times intradermal test may fail to become positive and also the previously positive result may become negative in terminal stages.[43] Cross reactors are greatest between histoplasmin, haplosporangin and blastomycin but these antigens show fewer cross reactions with coccidioidodin.

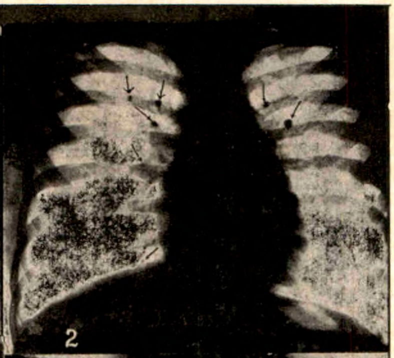
MATERIAL AND METHODS OF STUDY

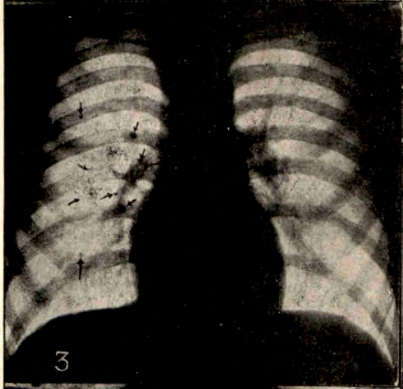
In this study, children and young adults examined were drawn from the following sources : (i) cases from the outpatient department of S. N. Hospital, (ii) medical students and student nurses of the Medical College and Hospital and (iii) school children from primary and higher boys and girls schools in Agra.

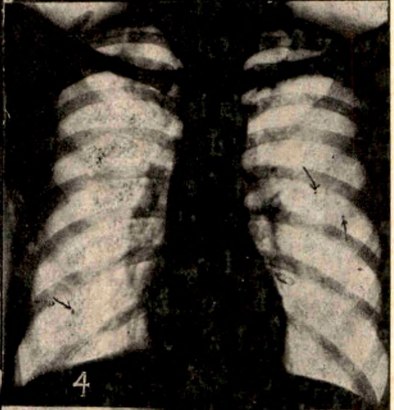
Cases were divided into various age groups as follows :— (i) under 5 years (ii) 5 to 9 years (iii) 10 to 14 years (iv) 15 to 19 years (v) 20 to 25 years and (vi) above 25 years.

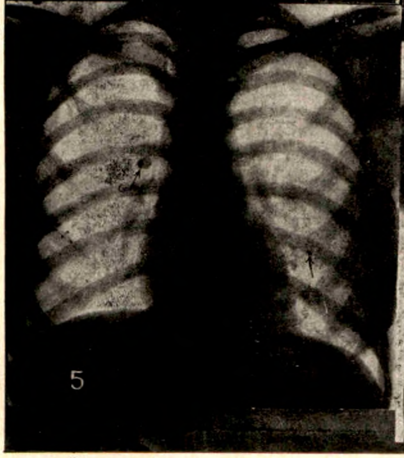
In each case skiagram of the chest was taken and the presence or absence of calcification in lung parenchyma or lymph nodes was noted. All the skiagrams were read by the radiologist to ensure uniformity of interpretation.











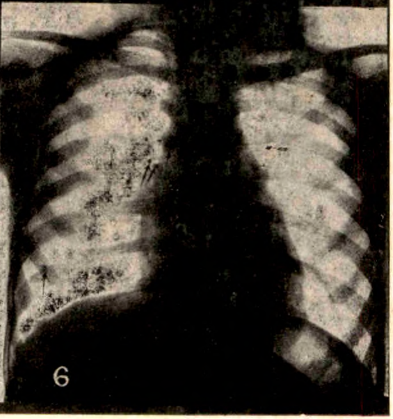


FIG. 1. Roentgenogram of the lungs showing paramediastinal calcification in a boy with histoplasmin negative and tuberculin positive reaction

FIG. 2. Disseminated pulmonaly calcification, strongly positive histoplasmin test, negative tuberculin test. Incidental chest X-ray findings in a boy. He had cough for three months.

FIG. 3. Disseminated pulmonary calcification in a boy. with both histoplasmin and tuberculin reactions positive.

FIG. 4. Roentgenogram of the lungs showing fan-shaped extensions and exaggerations of linear markings into the periphery. This boy had both tuberculin and histoplasmin positive reactions.

FIG. 5. Round areas of definite calcifications in the lung parenchyma in case with histoplasmin positive and tuberculin negative.

FIG. 6. Primary complex ype of calcification in a histoplamin sensitivə case.

The cutaneous tests were done in a battery of three, with a control of the diluting fluid. The tuberculin tests were performed with old tuberculin (Wellcome-Series T 2644F). A dose of 0·01mg. in 0·1 c.c. of fluid was given intradermally. Reaction with induration measuring 5 mm or more in diameter at 72 hours after injection was considered as positive. All other reactors were considered negative.

The histoplasmin used was kindly supplied by Dr. C. E. Roach of the Lilly Research Laboratories of Eli Lilly & Co. (CT-189). The antigen was supplied in concentrated form preserved with merthiolete 1 in 10,000. A dose of 0·1 c.c. of 1 in 1,000 dilution was used. Another lot (Lot H-42) was kindly supplied by Federal Security Agency of Public Health Service Washington D. C. The positive reaction consisted of erythema with induration and a surrounding zone of oedema measuring 0·5 cm or more in diameter after 48 hours. Erythema alone was not considered indicative of sensitivity.

Coccidioidin was kindly supplied by Dr. Charles E. Smith of the University of California, School of Public Health (Lot No. 29-31). The dose injected intradermally was 0·1 c.c. of 1 in 100 dilution. The results were read at 48 hours. Messers Cutler Laboratories of Berkeley, California also supplied us with a supply of coccidioidin (033-96).

Blastomycin concentrate (B-8564) was supplied by Dr. C. E. Roach of Eli Lilly & Co. Another supply was obtained from federal security Agency Public Health Service, Washington D. C. (Lot. B-7). The dose injected was 0·1 c.c. of 1 in 1,000 dilution. The results were read after 72 hours, the criteria of sensitivity being the same as with other antigens.

Tuberculin, histoplasmin and coccidioidin were all injected on the left arm at the same time by one person. The same person read the results by measuring the area of induration and erythema. In case where the area of induration was less than 5 by 5 mm (recorded as doubtful), the second strength solutions were used. Negative reactions had no erythema or induration. Blastomycin sensitivity was only tested in individuals who had lung pathology but were negative to tuberculin.

Since all the subjects were given tuberculin and histoplasmin at the same time, it was possible to note the types of reaction obtained with two antigens. No difference was observed and they could not be distinguished by their appearance.

Cultures were made in duplicate sets of (1) Sabouraud's agar slants (2) blood agar slants (3) beef infusion broth tubes (4) serum agar slants. One set was kept at room temperature and other was incubated at 37°C. The material cultured as a routine was sputum and blood in cases with positive histoplasmin reaction. In addition, all patients showing strongly positive reactions to histoplasmin had aspiration of sternal marrow done for culture and histologic examination.

RESULTS

Nine hundred and fifty-seven children and adults were tested with tuberculin, histoplasmin and coccidioidin. Sensitivity to blastomycin was tested in 91 cases only. Skiagrams of the chest were taken in nine hundred and forty-three cases.

Six hundred and ninety-six (72·7 per cent) reacted to 0·01 mg of old tuberculin. Nineteen cases (1·98 per cent) reacted to histoplasmin 1 in 1,000. None reacted to coccidioidin 1 in 100. Four hundred and six cases (43·1 per cent) had demonstrable pulmonary calcification. These results are shown in Table I.

TABLE 1. *The occurrence by number and percentage of sensitivity to tuberculin, histoplasmin and coccidioidin and the persence of intrathoracic calcification in Indian children and adults.*

Number examined	Tuberculin positive		Histoplasmin positive		Coccidioidin positive		Intrathoracic calcification	
	No.	Per cent	No.	Per cent.	No.	Per cent.	No.	Per cent.
957	696	72·7	19	1·98	0	0	406*	43·1

*X-ray not done in 14 cases.

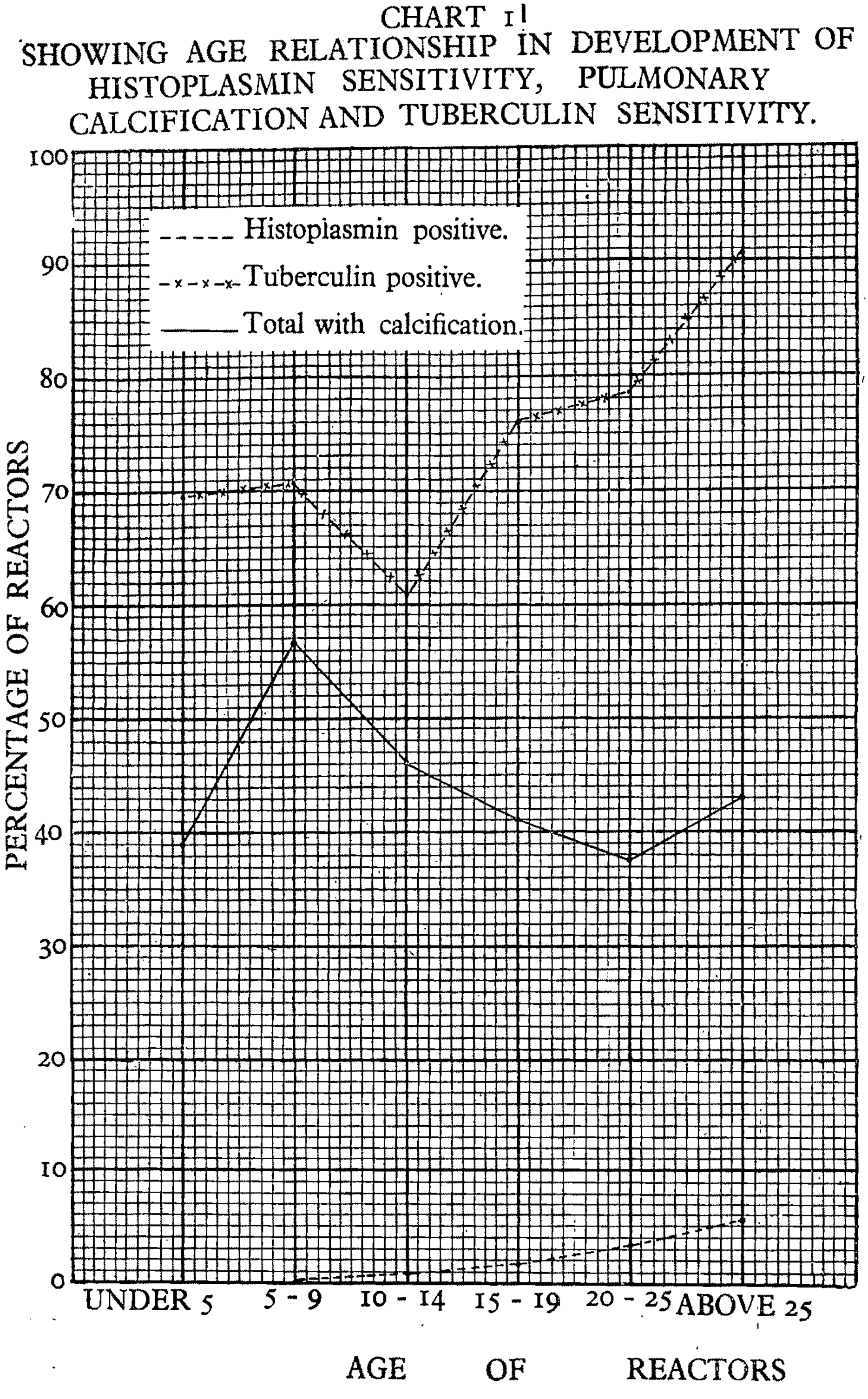


CHART I. Showing age relationship in development of histoplasmin sensitivity, pulmonary calcification and tuberculin sensitivity.

In Table II the age distribution of the children and adults in relation to their reactions is shown. These results show a rise in positive tuberculin reaction with the age except in age group of 10-14 years, where there is a fall (60·9 per cent). The highest percentage of tuberculin reactors are in the group of above 25 years (90·3 per cent). None of the cases below 10 years reacted to histoplasmin, while above 10 years there is a steady increase of histoplasmin reactors till the peak is reached in the age group of above 25 years (5·4 per cent).

TABLE II. *Number and percentage of Indian children and adults with calcifications : their reactions to histoplasmin and to tuberculin by age groups.*

Age	Number in age group	Tuberculin positive		Histoplasmin positive		Calcifications	
		No.	Per cent	No.	Per cent.	No.	Per cent.
Total ...	957	696	72.7	19	1·98	406 ϕ	43·1
Under 5 years ...	23	16	69·5	0	0	9	39·1
5-9 years ...	78	55	70·5	0	0	44	56·4
10-14 years ...	302	184	60·9	2	0·7	139	46·0
15-19 years ...	179	136	75·9	3	1·7	73*	41·0
20-25 years ...	282	221	78·3	9	3·2	101†	37·5
Above 25 years ...	93	84	90·3	5	5·4	40	43·0

*X-ray not done in 1 case.
†X-ray not done in 13 cases.
ϕX-ray not done in 14 cases.

From the above table it is noticed that highest incidence of pulmonary calcification (56·4 per cent) is in the age group of 5-9 years, while lowest (37·5 per cent) is in the age group of 20-25 years. There is not much change in other age groups. Another important point brought out by the table is the obvious difference between positive reactors to tuberculin and pulmonary calcification in different age groups. These results are graphically represented in Chart I.

Table III reveals the number and percentage of children and adults with calcification according to their reactions to tuberculin and histoplasmin.

TABLE III. *Number and percentage of Indian children and adults with calcifications according to their reactions to tuberculin and to histoplasmin.*

Type of reaction	No	Per cent of whole group	With calcifications	
			No.	Per cent of reaction group
Total ...	957	...	406	42·4
Tuberculin+Histoplasmin+ ...	15	1·56	11	73·3
Tuberculin+Histoplasmin− ...	681	70·1	301	44·2
Tuberculin−Histoplasmin+ ...	4	0·4	3	75·0
Tuberculin−Histoplasmin− ...	257	26·9	91	35·4

Eleven of fifteen cases (73·3 per cent), who reacted to both histoplasmin and tuberculin, had pulmonary calcifications. Three

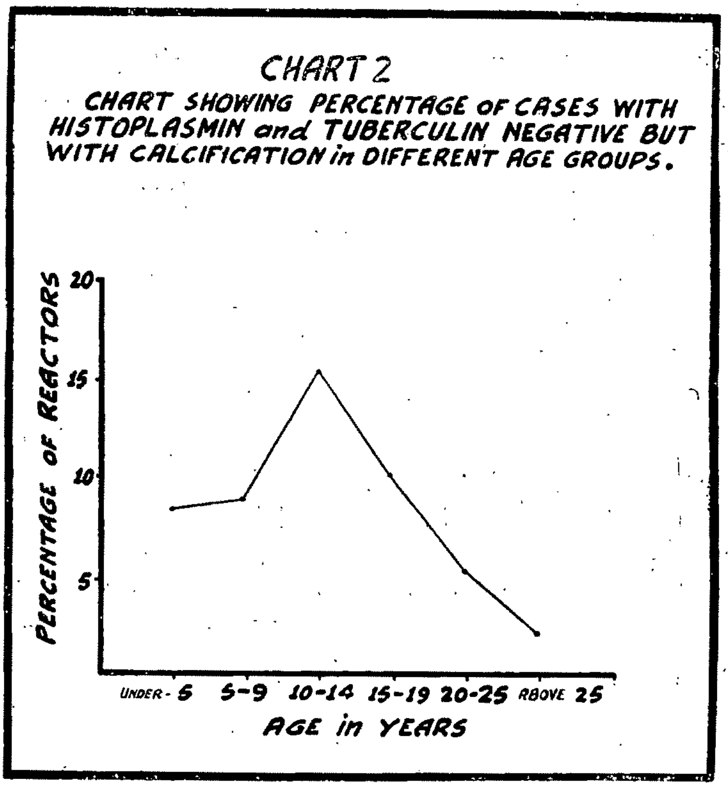


CHART II. Showing percentage of cases with histoplasmin and tuberculin negative, but with calcification, in different age groups.

of the four cases (75·0 per cent), who reacted only to histoplasmin, had pulmonary calcification. Three hundred and one out of six hundred and eighty-one cases (44·2 per cent), who reacted only to tuberculin, had pulmonary calcification, while ninety-one of two hundred and fifty-seven cases (35·4 per cent), who did not react to either antigen, had pulmonary calcifications. These cases according to age groups are graphically represented in Chart II.

Table IV represents the sensitivity to tuberculin and histoplasmin in four hundred and six children.

TABLE IV. *Histoplasmin and tuberculin reactions in 406 Indian adults and children having pulmonary calcifications.*

Type of reaction		Number		Per cent of all individuals with calcification	
Histoplasmin+Tuberculin+	...	11*		2·71	
Histoplasmin+Tuberculin−	...	3		0·74	
Subtotal Histoplasmin+	...		14		3·45
Tuberculin+Histoplasmin+	...	11		2·71	
Tuberculin+Histoplasmin÷	...	301		74·14	
Subtotal Tuberculin—	...		312		76·85
Histoplasmin − Tuberculin−	...	91		22·41	

*These individuals are duplicated to make subtotals correct.

Eleven of these cases (2·71 per cent) reacted both to histoplasmin and tuberculin, while only 3 cases (0·74 per cent) reacted to histoplasmin, the total histoplasmin reactions out of four hundred and six cases which had pulmonary calcifications being 14 (3·45 per cent). Three hundred and one cases (74·14 per cent) reacted to tuberculin only, the total tuberculin reactors being three hundred and twelve cases (76·85 per cent). Ninety-one cases (22·41 per cent) did not react to either antigen.

None of the cases reacted to coccidioidin while nineteen of nine hundred and fifty-seven cases tested (1·98 per cent) reacted to histoplasmin. Blastomycin sensitivity was tested in 91 children and adults. One of these cases (1·1 per cent) reacted to blastomycin (Table V).

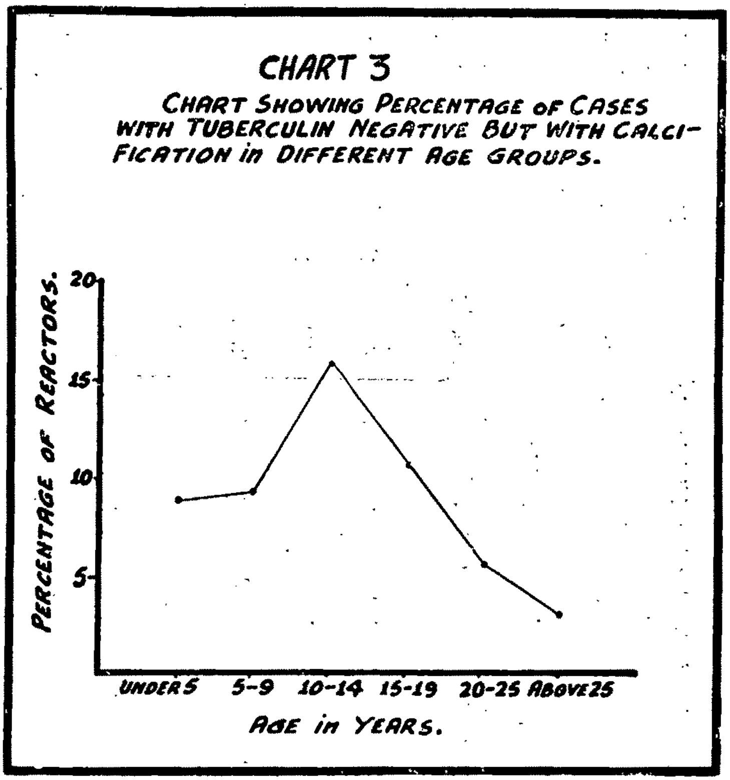


CHART III. Showing percentage of cases in the tuberculin negative but with calcification in different age groups.

Table VI shows sensitivity to histoplasmin according to sex. Seventeen of six hundred and seventy-five males (2·52 per cent) reacted to histoplasmin while only two of two hundred and eighty-two females (0·71 per cent) gave a positive reaction.

Table V. *Skin reaction to histoplasmin 1 in 1,000, blastomycin 1 in 1,000 and coccidioidin 1 in 100 in Indian children and adults by age groups.*

Age group	Sex		Total	Histoplasmin				Blastomycin				Coccidioidin			
				Positive		Negative		Positive		Negative		Positive		Negative	
	Male	Female		No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent
Under 5 years ...	14	9	23	0	0	23	100	0/4	0	4/4	100	0	0	23	100
5-9 years ...	61	17	78	0	0	78	100	0/4	0	4/4	100	0	0	78	100
10-14 years ...	214	88	302	2	0·7	300	99·3	0/12	0	12/12	100	0	0	302	100
15-19 years ...	134	45	179	3	1·7	176	98·3	0/18	0	18/18	100	0	0	179	100
20-25 years ...	185	97	282	9	3·2	273	97·8	0/49	0	49/49	100	0	0	282	100
Above 25 years	67	26	93	5	5·4	88	94·6	1/5	20	4/5	80	0	0	93	100
Total	675	282	957	19	1·98	938	98·02	1/91	1·1	90/91	98·9	0	0	957	100

TABLE VI. *Skinreaction to histoplasmin 1 : 1,000 according to sex.*

	Total number	Histoplasmin			
		Positive		Negative	
		Number	Per cent	Number	Per cent
Males ...	675	17	2·52	658	97·48
Females ...	282	2	0·71	280	99·29
Total ...	957	19	1·98	938	98·02

X-RAY INTERPRETATION

Nine hundred and fifty-seven children and adults had their chest X-rays taken. There was no specific pattern of pulmonary calcification in tuberculin or histoplasmin positive cases. The common findings were any one of the following : Paramediastinal calcification (Fig. 1), disseminated calcification where the calcified areas were either dense or fluffy (Fig. 2, 3), fuzzy fan-shaped extensions and exaggerations of linear markings into the periphery (Fig. 4), round areas of definite calcifications in the lung parenchyma 2·5 cms or more in diameter (Fig. 5). In a few cases, mulberry type apical focus in the higher age group and the primary complex in the younger age group were also seen (Fig. 6).

In the small number of histoplasmin positive cases that were found in the present study, the following patterns of calcification reported by Sontag and Allen[44] were not specifically seen in the present series : (1) Multiple in the parenchyma, often with calcifications present in upper lung fields and apices, (2) early in onset, frequently with initial lesion appearing before one year of age, the majority before 4 years of age, (3) preceded occasionally by a pneumonic infiltration early in the infection, (4) progressive with the development of new lesions and calcifications over a period of years.

The 'halo' primary focus, described as a specific lesion with respect to the diagnosis of histoplasmosis has not been found so in the present series, as also by previous workers.[1, 39] Furcolow, Mantz and Lewis,[21] and Silverman[39] have reported that the only roentgen finding which might be interpreted as the result of histoplasmosis infection with any degree of probability is disseminated intrathoracic calcification. This has been the finding in the present study also.

DISCUSSION

42·4 per cent of persons studied in the present series had pulmonary calcifications. Of the total number with calcifications, 23·15 per cent did not react to tuberculin. In many of these cases clinical and laboratory examinations did not reveal a tuberculous etiology. What then is the cause of such pulmonary calcifications—tuberculosis ? pulmonary mycosis ?

The problem of differential diagnosis of tuberculous and mycotic etiology of pulmonary calcification has been accentuated recently due to two factors, first by the current practice of roentgenological survey of populations, which results in the demonstration of pulmonary lesions in many individuals not clinically ill, and secondly by the routine discharge roentgenograms of army personnel since World War II. The latter have shown a number of men who have developed pulmonary calcifications which were not present at the time of their joining the service. During the last decade large number of studies have been undertaken by various workers to elucidate the significance of these clacifications. It would seem from these studies that majority of these in United States were probably not due to tuberculous infection but rather to a benign pulmonary mycosis resulting in healed calcified nodules.[8] Palmer[34] has reported that in some of the states in U. S. A. distribution of tuberculosis is not at all closely correlated with the frequency of calcification. This has been further supported by reports of other workers.[15, 22, 28, 32]

These observations led to a search for a disease other than tuberculosis as a cause of pulmonary calcification in man. Olson, Wright and Nolan[32] considered ascariasis but were unable to prove it. Cox and Smith[10] in 1939 and Aronson *et al.*[2] in 1942 concluded as a result of their studies in certain localities of California that coccidioidomycosis was responsible for considerable amount of calcification in these localities. Smith[41] in 1943 stated that majority of pulmonary calcification in western Ohio was due to histoplasmosis. Similar were the findings of Christie and Peterson[7, 8, 9, 37] in Tenneessee.

Similar problem has confronted us in India when it has been known for some time that pulmonary calcifications are present in a good number of individuals which gave a negative reaction to tuberculin. The recent mass B. C. G. vaccination campaign definitely established this fact. The present study is the first systematic investigation in this country to find out the possible mycotic origin of these non-tuberculous pulmonary calcification.

However, there are still workers who believe that essentially all pulmonary calcification is due to tuberculosis and explain a negative tuberculin reaction in such cases by assuming that sensitivity to tuberculin has been lost with the healing of the lesion or due to other causes. This explanation is not acceptable on the basis of previous studies[34] and also the present findings, where many of the cases occurred in children. Dahlstrom[12] in his studies of repeated tuberculin tests found an extremely low rate of reversion to negativity in persons who react to a low dose of tuberculin.

Since the publication of reports of Aronsen *et al.*[2] Smith[41] and Christie and Peterson, [7, 8, 9, 37] stressing *Coccidioides immitis* and *Histoplasma capsulatum* as important pulmonary pathogens, definite suspicion was aroused that many of the lesions in this country are mycotic in origin. Attempts to establish diagnosis in such cases were made and included procedures intended to demonstrate the presence of fungi. In most of the cases studied there was no sputum or other pathological exudate which could be examined. Immunological methods were then the only sheet anchor and were thus adopted for diagnosis. Unfortunately their reliability as diagnostic procedures in mycotic infections is questionable. On the one hand antibodies may not develop, and on the other cross reactions between different fungal antigens have been definitely demonstrated.

Immunological data may therefore be absolutely unreliable in the diagnosis of a given case. A positive skin reaction may however be of immense value in yielding interesting epidemiological information in the sense that it may indicate sensitization acquired as a result of subclinical or mild infection. If these reactions are seen in present or past residents of an endemic area, the significance is definite. What is their significance, however, if the reactions occur in individuals who have had no known exposure to coccidioidis or histoplasmoomycosis ? To be more pointed, what value is to be attached to nineteen positive reactors to histoplasmosis in the present study, which are derived from western region of Uttar Pradesh. The possibilities are : (1) This is an endemic area not previously recognized with a possibility of the existence of a benign infection with *Histoplasma capsulatum* (2) These are aberrent reactions, the significance of which is not known; may be these are cross reactions due to some fungus other than histoplasma, coccidioides or blastomyces or (3) the lots of antigen used gave non-specific reactions. This is however not correct because the lots were adjudged suitable for use by the donors.

The results so far as coccidioidomycosis and blastomycosis are concerned are essentially negative, since no reactors to coccidioidin were found and only one reacted to blastomycin. With histoplasmosis the problem is a little different because nineteen cases reacted to histoplasmin. Any inference of a relationship between a reaction to the histoplasmin antigen and infection with histoplasma depends upon the specificity of the histoplasmin test.

Little definite knowledge on this point is as yet available. However, it is apparent from the studies till date that a positive histoplasmin test even in young persons has no significance as an indicator of active disease. None of the nineteen cases which gave a positive reaction had any evidence of clinically significant infection. Similarly patients with advanced proved histoplasmosis have given negative reactions.[46], [47], [48] Its value as an exclusion test is probably the same as that of tuberculin is in mild acute, subacute and chronic infection.

The specificity of histoplasmin reaction has recently been disputed by Emmons, Olson, and Eldridge[30] by the results of their experimental work on animals. They have shown that histoplasmin gives a positive reaction in animals experimentally infected with *Blastomyces dermatitidis* or *Haplosporangium parvum* and *Coccidioides immitis*, though much less frequently in the last infection. If their experimental findings are true for natural infection in human beings, then one might infer that histoplasmin gave frequent non-specific reactions, and as such may serve as an indicator of sensitisation to various fungi. Christie and Peterson[9, 11] and Palmer[22] have indicated such a possibility in human infections. A positive histoplasmin reaction in areas not endemic for histoplasmosis might therefore mean sensitisation by some agent having an antigenic complex closely related to that of histoplasmin.[10] Christie and Peterson[10] however finally concluded that there is less tendency for cross reactions to occur in children and young adults than in experimental animals as judged by the failure to get any false reactions with coccidioidin. They have made a further rather important observation that the reactions observed are truly allergic phenomenon, reactions to some antecedent infection, rather than simple reactions to non-specific irritants contained in the preparation.

The present study has not proved a direct casual relationship between pulmonary calcifications and histoplasmosis, as it has not been possible to culture the fungus in any of the cases. Further light on the etiology of pulmonary calcifications may be thrown by the studies on cross sensitivity among fungus antigens and infections. It is more than probable that an etiologic agent other than tubercle bacillus responsible for pulmonary calcifications and histoplasmin sensitivity may be found.

These studies serve an important purpose in pointing out that minimal and healing pulmonary lesions with calcification should not be labelled as tuberculous without employing tuberculin tests or demonstration of tubercle bacilli. Possibility of fungus infection should always be kept in mind. In the present state of our knowledge, it seems best to interpret the histoplasmin reaction as evidence of previous infection with *Histoplasma capsulatum* or an immunologically related organism.

SUMMARY

A study of 957 Indian children and adults with regard to roentgenograms of their chests and the cutaneous sensitivity to histoplasmin, tuberculin, coccidioidin and blastomycin sensitivity is presented.

The subject of the concurrence of histoplasmin, blastomycin and coccidioidin sensitivity and pulmonary calcification is reviewed.

The relationship of age and sex in the development of tuberculin sensitivity, histoplasmin sensitivity and calcification has been presented.

The significance of histoplasmin sensitivity in Indians, as revealed by the present study has been discussed. Even if the interpretations of the specificity of the histoplasmin test is correct, histoplasmosis is not a common infection in this part of the world.

It is suggested that it should be included by the radiologist in the differential diagnosis of pulmonary inflammatory diseases.

The absence of cutaneous sensitivity to coccidioidin in 957 subjects, 42·4 per cent of whom were found to have pulmonary calcification, would seem to rule out the possibility of coccidioidomycosis playing any significant role in the development of non-tuberculous pulmonary calcifications in residents of Uttar Pradesh.

There is an obvious discrepancy between the finding that 19 cases reacted to histoplasmin and to the fact there has been no recognized cases of histoplasmosis in Uttar Pradesh. Further studies are in progress to determine whether histoplasmin sensitivity is due to cross reactions in patients who have had infections with antigenically related fungi for or to infection with *Histoplasma capsulatum*.

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REFERENCES

1. Absher, W.K., & Cline, F., Jr. 1949. *Am. Rev. Tuber.* **59** : 643.
2. Aronson, J.D., Saylor, R.M., & Parr, E. I. 1942. *Arch. Path.* **34** : 31.
3. Bass, H.E. 1950 *J. Amer. med. Assoc.,* **143** : 12.
4. Bass, H. E., Kooperstein, S, I., Friedman, M. M. & Kastlin, G. H. 1946. *Dis. of Chest.,* **12** : 1.
5. Bass, H.E., Schomer, A. & Berke, R. 1948. *Arch. Int. Med.* **82** : 519.
6. Beadenkopf, W.F., Loosli, C.G., Lach, H. Rice, F.A. & Slattery, R.V. 1949. *Pnub. Health Rep.,* **64** : 17.
7. Christie, A. & Peterson, J.C. 1945. *Am. J. Pub. Health,* **35** : 1131.
8. Christie, A. & Peterson, J.C. 1946. *J. Pediat.,* **29** . 417.
9. Christie, A. & Peterson, J.C. 1946. *J. Amer. med. Assoc.,* **131** : 658.
10. Cox, A.J. & Smith, C.E. 1939. *Arch. Path.,* **27** : 717.
11. Craddock, W.L. 1950. *Mississ. Val. Med. J.,* **72** : 15.
12. Dahlstrom, A.W. 1940. *Am. Rev. Tuberc.,* **42** : 471.
13. Davis, B.F. 1911. *J. Infect. Dis.,* **8** : 190.
14. Davis, H.V. & Neff, F.C. 1946. *Am. J. Dis. Child.,* **71** : 171.
15. Dearing, W. P., Olson, B. J , Self, L. R. W., & Baggett, M. W. 1941. *Nat. Tuberc. Assoc. Thirty-seventh Ann. Meeting.*
16. Dickson, E.C. & Gifford, M.A. 1938. *Arch. Int. Med.,* **62** : 853.
17. Edward, L.B., Lewis, L. & Palmer, C.E. 1948. *Public Health Rep.,* **63** : 1569.
18. Emmons, C.W., Olson, B.J. & Eldridge, W.W. 1945. *Pub. Health. Rep.,* **60** : 1383.
19. Ferebee, S.H., & Furcolow, M.L. 1947 *Public Health Rep.,* **61** : 1132.
20. Furcolow, M.L., High, R.H. & Allen, M.F. 1946. *Pub. Healh Rep.,* **61** : 1132.
21. Furcolow, M.L., Mantz., H.L. & Lewis, I. 1947. *Pub. Health Rep.,* **62** : 1711.
22. Gass, R.S., Gauld, R. L. Harrison, E.F., Stewait, H.C. & Williams, W.C. 1938. *Am. Rev. Tuberc.,* **38** : 441.
23. Goddard, J.C., Edward, L B. & Palmer, C.E., 1949. *Pub. Health Rep.,* **64** : 820.
24. Groover, M.F., Jr., Cleve, E.A., Bornstein, S., Rice, A.G., Galloway, H.F. & Macaluso, C.P. 1947. *Arch Int. Med.,* **80** : 496.
25. High, R.H., Zwerling, H.B. & Furcolow, M.L. 1947. *Pub. Health Rep.,* **62** : 20.
26. Long, E.R. 1926. *J. Amer. med.Assoc.,* **87** : 1441.
27. Long, E.R. & Stearns, W.H. 1943. *Radiology.,* **41** : 144.
28. Lumsden, L.L. & Dearing, W.P. 1940. *Am. J. Pub. Health.,* **36** : 219.
29. Martin, D.S. & Smith, D.T. 1931. *Am. Rev. Tub.,* **39** : 488.
30. Martin, D.S. & Smith, D.T. 1936. *J. Lab. & Clin. Med.,* **21** : 1289.
31. Mc Leod, J.H., Emmons, C.W., Ross, S. & Burke, F.G. *J. Pediat.,* **26** : 275.
32. Olson, B.J., Wright, W. H. & Nolan, M.O. 1941. *Pub. Health Rep.,* **56** : 2106.
33. Pack, R. L. Martin, D.S., & Hausar, C.R. 1940. *J. Immunol.,* **38** : 449.
34. Palmer, C.E. 1945. *Pub. Health Rep.,* **60** : 513.
35. Palmer, C.E. 1946. *Pub. Health Rep.,* **61** : 475.
36. Parson, R.J. & Zarafonetis, C.J.D. 1945. *Arch. Int. Med.,* **75** : 1.
37. Peterson, J.C., & Christie, A. 1948. *Am. Rep. Tuberc.,* **57** : 361.
38. Prior, J.A. & Allen, M.F. 1949. *Pub. Health Rep.,* **62**. 1608.
39. Silverman, F.N. 1950. *Am. J. Roentgenology & Rad. Therapy,* **64** : 5.
40. Smith, C.E. 1943. *M. Clin. North America,* **27** : 790.
41. Smith, C.E., Beard, R, R., Rosenberger, H.G. & Whiting, E.G. 1946. *J. Amer. med. Assoc.,* **132** : 883.

42. Smith, C.E., Saito, M.T., Beard, R. R., Rosenberger, H. G. & Whiting, E. G. 1949. *Am. J. Pub. Health*, **39** : 722.
43. Smith, D.T. 1949. *J. Amer Med. Assoc.*. **41** : 1223.
44. Sontag, L.W. & Allen, J.E. 1947. *J. Pediat.*, **30** : 657.
45. Stober, A.M. 1914. *Arch. Int. Med.*, **13** : 509.
46. Stokes, J.H., Beerman, H. & Ingraham, N.R. 1950. *Am. J. Med. Sci.*, **220** : 435.
47. Ziegler, E.E. 1946. *Ann. Int. Med.*, **24** : 1073.
48. Zwerling, H.B. & Palmer, C.E. 1946. *Radiol.*, **47** : 59.

CROHN'S DISEASE OF THE ILEOCAECAL REGION*

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Crohn's disease or cicatrising enteritis is an uncommon, nonspecific, inflammatory disease, diagnosed by excluding tuberclousis, sarcoidosis and other granulomas like lymphogranuloma venereum. Continental writers have named it *ileitis stenosans* or *ileitis ulcerosa.* Recognition of the widespread involvment of the intestines (small or large) has led to terms like cicatrising enteritis or regional enteritis. My interest was aroused in the subject when a lump in connection with the caecum, treated by hemicolectomy on the supposition that it was a case of ileocaecal tuberculosis, proved on histological examination to be Crohn's disease. I did other resections for similar types, which also proved to be cases of Crohn's disease. In several others I did short-circuiting operations with section of the ileum and the patients showed marked improvement, with reduction in the size of the lump. A list of fifteen cases, which were followed up, is given in this paper. This paper is presented with the object of emphasizing that lumps, which are diagnosed as hypertrophic ileocaecal tuberculosis, are not always tubercular and that some of them may really be Crohn's disease.

Eight of my cases were diagnosed by histological examination, four after the removal of the mass by hemicolectomy and one of small intestinal stricture after its resection. The diagnosis of the rest of the cases mentioned here could not be confirmed except in three cases. From two of these a gland was removed for biopsy, which showed granuloma and hyperplastic adenitis. In another case hard nodules in the fatty mesentry showed granulomas of cicatrising enteritis.

The earliest case was recorded in 1806 and until recently there have been other sporadic case reports. In 1909 Braun[1] described 7 cases with inflammatory masses in the colon having the appearance of a neoplasm. In 1913 Dalziel described 9 cases of chronic interstitial enteritis with thickening of the small and large intestines. Moschowitz and Wilensky[9] were the first to point out that many cases of nonspecific granuloma had been incorrectly diagnosed as hyperplastic tuberculosis. Hadfield[7] *in* 1932 described certain histological features, which he regarded being specific of Crohn's disease.

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*Paper read at the Association of Surgeons of India, Calcutta, 1951.

Warren and Summer's statistics[14] of the sites of lesion are as follows :—

Ileum	93%	Appendix	24%	Proximal colon	15%
Skip areas	9%	Jejunam	4%	Distal colon	1%

and those of George Armitage and Wilson are :—

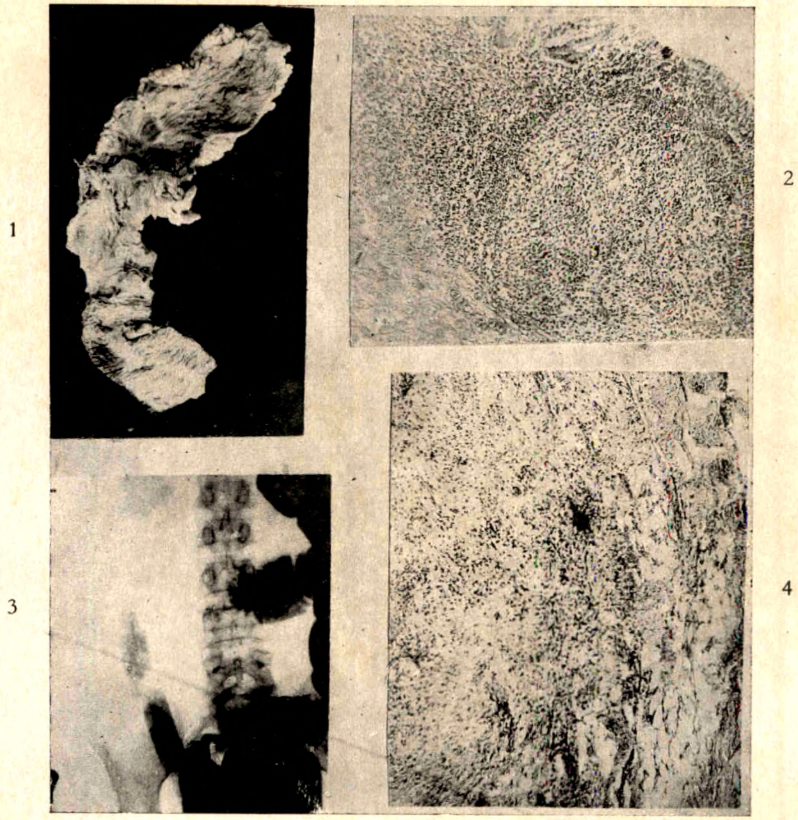
Ileum	88%	Colon	38%	Jejunum	3%

Cicatrising enteritis usually involves a short segment of terminal ileum and hence the term terminal ileits ; but the jejunum, caecum and ascending colon are also affected in about 10% of cases. There are no reports of the oesophagus, stomach or duodenum having been involved.

The usual age incidence reported is under 40. Wilson[6] reported an average of 31 years, oldest being 69. Crohn described a preponderance in males in the ratio 2 : 1. Warren[14] reports equal number of cases in males and females. In my small series of cases the age group shows a preponderance in females as follows :

Table I. Cases where resection of bowel was done in the last one year.

	Males	Females	Total	Results
1. Sex	3	3	6	
2. Age	30 Yrs.	17 Yrs.		
	25 „	25 „		
	25 „	35 „		
3. Lump in right iliac fossa with signs of chronic obstruction	2	2	4	
4. Without lump	1	1	2	
5. Hemicolectomy	2	2	4	Marked improvement
6. Resection of ileum		2	2	



Case No. IV. Name—H. C. K. 20 yrs. Hindu male. 1. Resected specimen of caecum and ascending colon. 2. Photomicrograph of section, showing granuloma of cicatrising enteritis and giant cells. *Case No. V.* Name—Mrs. D. 40 yrs. Hindu female. 3. Barium enema showing filling defects of ascending colon and hepatic flexure of colon. 4. Photomicrograph of a section of fatty nodules and a piece of intestine. Ileotransverse colostomy was done.

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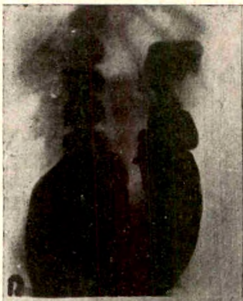

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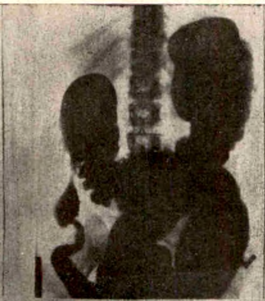

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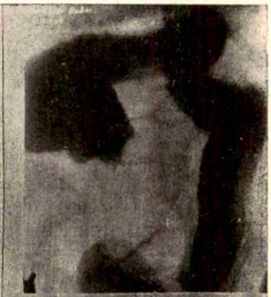

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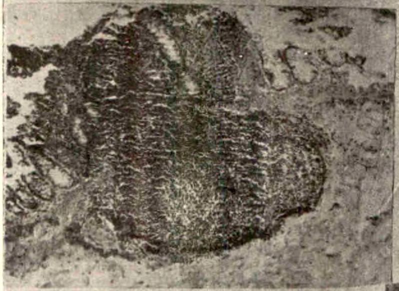

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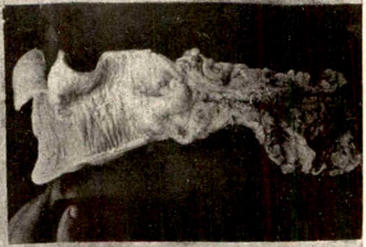

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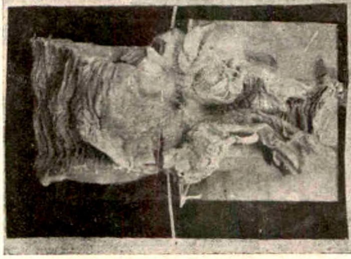

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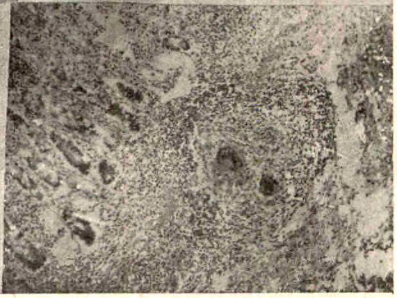

Case No. VI. Name—R. B. 15 yrs. Hindu male. Case of fistula with stricture of splenic flexure of colon. 5. Barium enema showing stenosis of splenic flexure. 6. A typical X-ray picture of stricture of ascending colon, caecum and terminal ileum in a case of Crohn's disease of ileocaecal region.

Case No. I. Name—S. D. 17 yrs. Hindu female. 7. Barium enema showing filling defect of caecum. 8. Resected specimen of caecum and ascending colon. 9. Photomicrograph of section of granulomatous lesion. *Case No. II.* Name—K. M. 30 yrs. Hindu male. 10. Resected specimen of caecum and ascending colon. 11. Photomicrograph of section, showing giant cells of Crohn's granuloma.

Table II. Cases where short-circuiting was done and followed up in the last one year.

	Males	Females	Total	Results
1. Sex	1	6	7	
2. Age	30 Yrs. 5 between 20 & 24 Yrs.	45 Yrs.		Marked improvement in symptoms with reduction in the size of lump in all cases.
3. Lump in the right iliac fossa with signs of chronic obstruction	1	4	5	
4. Without lump	–	2	2	

Table III. Cases with fistulae following opening of abscesses : Males 2 aged 30 years and 50 years respectively.

CLINICAL COURSE

Crohn and others have described 4 clinical types :—

1. Acute intra-abdominal manifestation resembling cases of acute appendicitis—8 cases reported by Warren. Of these eight, five were acute exacerbations of chronic cases(25%).
2. Subacute phase and ulcerative enteritis—4% (Warren).
3. Those in stenotic or chronic phase—93% (Warren).
4. Cases of persistent and intractable fistulae—9% (Warren)

It is the chronic or stenotic group which I report here. Eight of these were diagnosed histologically as cases of cicatrising enteritis by histological examination of specimens of bowel or glands. They complained of periodic cramp-like pains in the abdomen with borborygmi and visible peristalsis. There was occasional looseness of bowels but constipation and inability to pass wind was the main symptom. Four patients complained of alternating constipation and diarrhoea. All cases except four had palpable lumps in the right iliac fossa, which were tender on deep pressure. The lump was more or less well defined and slightly movable. Sometimes the distended loop of bowel proximal to the obstruction was tense and hard but disappeared as the peristalsis passed off. In a few cases there was some amount of persistent distension because of sub-acute obstruction. There was no history of contact with tubercular cases. A familial incidence was not noted. The duration of the attacks of pain ranged between 2 to 4 years. One patient had symptoms for 8 years, having attacks of pain with lump in the right hypochondrium. This lump used to disappear in a day or two after the attack of pain had subsided. She did not have any palpable lump in the right iliac fossa. The whole of the ascending and transverse colon was affected and ileosigmoicolostomy had to be performed for the relief of obstruction. She has remained well since the operation.

One case was suspected to be one of acute terminal ileitis when appendicectomy was done. Later she came as a case of obstruction after about 10 months, when resection of about 6 inches of ileum had to be done. She has been well since then.

A palpable mass in the right iliac fossa has been described in 38% of cases, with tenderness depending upon the acuteness of the condition.

Five cases who had lumps in the right iliac fossa and on whom only ileotransverse-colostomy was performed, improved more rapidly than those treated by resections of the colon. In these cases the lump disappeared in about a month's time with complete relief of symptoms. There was no evidence of tubercles on the surface of the caecal lumps or ileum in any of my cases, nor of caseation of the mesenteric glands as far as could be made out. The histological examinations of the pericolic glands and a piece of terminal ileum showed evidence of granuloma and cicatrising enteritis in two cases.

Persistent and intractable fistulae, which have been described in 9% of cases, are frequently a manifestation of Crohn's disease after an operation for appendicitis or draining of an abscess. I had a case of persistent fistulae resulting from incision of ileac abscesses on both sides. These fistulae discharged faecal matter for several days. He developed an ischiorectal abscess and fistulae *in ano.* Ultimately he developed secondary anaemia and died of an intercurrent illness. He had developed hard inflammatory lumps in the regions of the colon on both sides following an attack of acute abdomen. Another case of faecal fistulae developing after incision of left lumbar abscess was suspected to be due to Crohn's disease of left splenic flexure, which showed stricture on barium enema. The fistulae closed by themselves and the patient could pass normal stools. He may need an operation of ileosigmoidostomy if resection is not done. There is no lump palpable in the abdomen. The case

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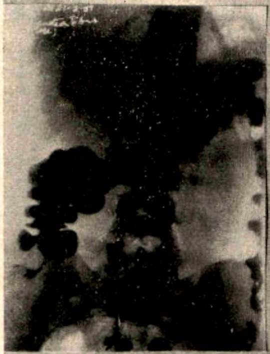

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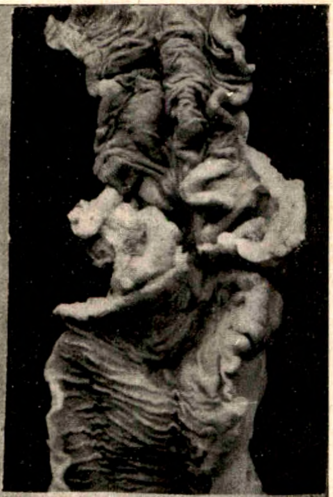

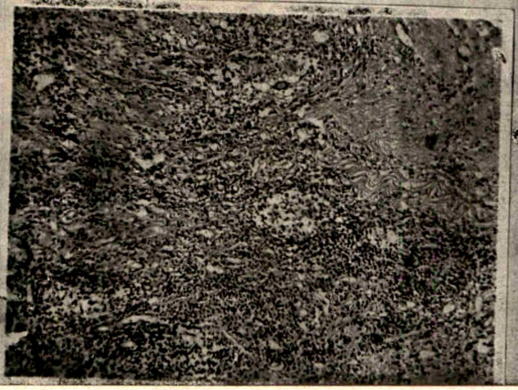

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Case No. III. Name—Prem Vati. 24 yrs. Hindu female. 12. Barium enema showing filling defect of caecum. 13. Photo of resected caecum and ascending colon. 14. Photomicrograph of section, showing granuloma and giant cells.

with small intestine stricture had no palpable lump but came with only symptoms of chronic obstruction. On laprotomy a mass of adherent ileum was found in the pelvic cavity attached to the posterior wall of the uterus. The resected piece of ileum could hardly admit a probe. About 5 to 6 inches of ileum was resected and side to side anastomosis was done. Two other cases also had no lump palpable in the right iliac fossa. There was only X-ray evidence of stenosis in the colon and caecum. Histological examination of the excised caecum and ascending colon revealed cicatrising enteritis in one, and granulomatous structure in transverse colon in another, where ileo-signiodostoromy was done.

Differential diagnosis.—Ileocaecal tuberculosis. My mistake in the diagnosis of hyperplastic ileocaecal tuberclosis was detected after receiving the pathological reports of the resected specimens. Cases with lumps in the right iliac fossa when first seen were diagnosed as tubercular before Crohn described the condition affecting the ileocaecal region. The differentiation is purely histological or bacteriological and cannot be made clinically or on naked eye appearance. Crohn's disease was in the past erroneously regarded as a tubercular condition. The differentiation between the two is indeed very difficult. The diagnosis rests purely on expert histological findings, *viz.* absence of caseation, absence of tubercle bacilli and negative report after guinea-pig inoculation. Microscopically the presence of granulomas with giant cells provides the main source of difficulty in distinguishing the two diseases. The granulomas of Crohn's disease have a disorderly appearance with indistinct cell boundaries, the endothelial cells are mixed with lymphocytes, the giant cells being scalloped and irregular in size and shape. There is no caseous necrosis and tubercular bacilli are absent. Warren[14] is of the opinion that cases which were once called hyperplastic tuberculosis should be labelled as Crohn's disease and that the former term is obsolete. The same histological findings as those of granulomas of Crohn's disease have recently been reported by V.S. Manglik and S.C. Misra[8] in the resected specimens of bowel and glands in suspected cases of primary ileocaecal tuberculosis.

2. The resemblance between a granuloma of cicatrising enteritis and sarcoid has been noted by many who consider them morphologically indistinguishable. Warren says that the granuloma of sarcoid are larger and more numerous without lymphocytic infiltration. The spiculated bodies seen in the giant cells of sarcoid were absent in his series of cases of cicatrising enteritis.

Indentical cellular aggregations have been observed in diverticulitis, cancer and lymphoma of intestines.

3. Ulcerative colitis and lymphogranuloma venereum of colon have to be distinguished from Crohn's disease.

The former involves the ileum in 25% of cases and clinically may be difficult to distinguish from cicatrising enteritis. The thickening and fibrosis of bowel following amoebic dysentry is less than that found in cicatrising entritis. Frie's test will aid the diagnosis of lymphogranuloma.

4. Talc granuloma caused by excess of powder on gloves at laprotomy may produce an appearance indistinguishable from regional ileitis. Microscopically doubly refractile crystals surrounded by epitheloid or giant cells are found.

5. Cases of Crohn's disease with fistulae should be distinguished from actionmycosis and the characteristic discharge confirms the diagnosis.

It is not possible to enter into the details of etiology here. Most authorities have considered it to be due to infection of unknown etiology. Bacteriological studies have however failed to reveal any specific agent. Various bacteria of doubtful pathogenecity have been isolated such as anaerobic streptococci, *Bacillus proteus* and *Aerobacteria aerogenes*. Felson considers cicatrising enteritis and ulcerative colitis are related to bacillary dysentry.

Pugh's[12] contention is that the absence of a known etiological agent is a prerequisite to the diagnosis of cicatrising enteritis. The microscopic picture is that of a response to lipid, as is produced experimentally by injection of animal oils according to Pinkerton. There is swelling and proliferation of lymphatic endothelium in intestine and lymph-nodes, causing lymphatic obstruction and oedema. Granulomas containing giant cells are formed in the intestinal wall, mesenteric lymphnodes and liver which slowly hyalanise without necrosis. Fibrosis and muscular hypertrophy of the intestine occur in subacute and chronic stage.

Treatment.—Resection is the surgical treatment of choice and it is said to cure 83 to 85% of cases.[11,13] The five cases where I performed resection of intestines improved to a great extent but complained of inability to pass flatus and had loose motions two or three times in a day. One of the cases did not improve as well as expected while the others had great relief in symptoms. Seven other cases who had short circuiting (ileocolostomy with division of the ileum or exclusion) improved considerably and the lumps disappeared almost completely after a month or two of the operation. The stools were well formed even in the cases of ileosigmoidostomy. Side-tracking operations are successful in 49 to 87% of cases.[14] The results of short circuiting with exclusion were better than with resection in my series of cases.

Recurrence is a feature of this disease and becomes apparent clinically as late as 10 years after operation. Rockers (quoted by George Armitage,[6]) says that good results may be anticipated in more than 50% of resections. In George Armitage and Wilson's[6] series the recurrence occurred 12½ years, 5 years 4 years and 1 month respectively after operation in 4 of their cases. W. F. Nickel[10] reports that the prognosis is poor in cases where more than 150 cm. of bowel is involved or resected, as such cases are more prone to recurrences.

SUMMARY

1. Eight cases of histologically proved Crohn's disease are recorded. Resection of the ilecaecal lump was done by right hemicolectomy in four cases, and resection of small intestine in two cases.

2. Six other cases of caecal lumps suspected to be Crohn's disease were treated by the operation of short-circuiting and section of the terminal ileum with better results than resection. One of these cases was diagnosed by histological examination of a gland and another by the examination of hard nodules in the mesentry of the colon showing granulomas of cicatrising enteritis.

Two cases without palpable lumps were treated by ileosigmoidostomy as the disease was affecting the transverse colon.

3. Two cases of suspected Crohn's disese with fistulae were met with.

4. Difficulties are discussed of differentiating Crohn's disease from tuberculosis when an ileocaecal lump is palpable.

5. A few essential points in pathogenesis and treatment are described.

I am thankful to Dr. P.N. Wahi, M.D., M.R.C.P., (London), Pathologist, for the histological reports and Dr. P.K. Haldar, D.M.R. & E., etc. for the X-ray photographs. I am also thankful to Dr. H.N. Bhatt, Superintendent, for permission to utilize the case reports.

REFERENCES

1. Braun, H. 1920. Ueber entzundliche Geschwulste am Darm. *Deut.Z.chir.*, **12** : 211-273.
2. Cambe C. Saunders, W.A., 1813. A singular case of stricture and thickening of ileum. *Med. Tr. R. College Phys. London*, **4** : 16-21.
3. Crohn, B.B., L. Ginsberg & G.D. Oppenheimer. 1932. Regional ileitis—a pathological and clinical entity. *J. Amer. med. Assoc.*, **99** : 1323.
4. Dalziel, T.K. 1913. Chronic interstitial enteritis. *British med. J.*, **2** : 1068-1070.
5. Garlock, J.H. & B.B. Crohn. 1945. Apprisal of results of surgery in treatment of regional ileities. *J. Amer. med. Assoc.*, 205.
6. George Armitage & Wilson. 1950. Crohn's disease. *British J. Surg.*, **27** : 182.
7. Hadfield, G. 1939. The primary histological lesion of regional ileitis. *Lancet*, **2** : 773-775.
8. Manglick, V.S. & S.C. Misra. 1952. Primary intestinal tuberculosis. *J. Indian med. Assoc.*, p. 12.
9. Moschowitz, E. & A.O. Wilensky. 1923. Nonspecific granulomata of intestine. *Amer. J. med. Sci.*, **166** : 48-66.
10. Nickel. W. F. 1950. Ulcerative colitis and cicatrising enteritis. *Surgical Clinics of America*. pp. 546-553.
11. Pratt, G. & L. K. Ferguson. 1937. Cicatrising entiritis. *Amer J. Surg.*, **35** : 607-608
12. Pugh, H.L. 1915. Regional enteritis. *Ann. Surg.*, **122** : 845-861.
13. Rhoads, J. E. 1939. The management of regional ileitis and certain other ulcerative lesions of intestines. *Pennsylvaania med. J.*, **42** : 1050-1053.
14. Warren & Summers. 1948. Regional ileitis as a pathological entity. *Amer. J. Pathol.*, **24** : 475,
15. Wirts, C.W. & B.B.v. Lyon. 1941. The incidence of regional ileitis., *Amer. J. Digestive Dis.*, **8** : 246-248.

ALLOTROPIC MODIFICATION OF A SUBSTANCE AND THE JOSHI EFFECT

By G. N. PENDSE, *Demonstrator in Physics, Holkar College, Indore.*

Certain substances like Phosphorus and Sulphur are known to exist in various allotropic modifications. When a substance changes from one modification to another, the properties get modified. Vapour pressure of the vapour in contact with the solid phase alters and complexity of the molecule affected. Joshi (1, 2, 3) has established that in gases and vapours of simple molecules negative Joshi Effect is widespread than the positive effect. Polyatomic gases favour positive Joshi Effect. Hence it was of interest to extend the studies of Joshi Effect in systems which show allotropy. It is known (4) that red phosphorus is produced when an electric discharge is sent through the vapours of ordinary phosphorus and hence an attempt was made to study the changes associated with it and if possible to relate Joshi Effect to the changes consequent to the conversion of one variety into another.

EXPERIMENTAL.—A small quantity of pure yellow phosphorus dissolved in carbon-tetra-chloride was filled into a pyrex glass. Siemens Ozoniser (manufactured by Biswas & Co. Calcutta) and sealed at pressure of a few mms. of mercury and temp. 15°C. The phosphorus vapour was in contact with the solid phase. The general assemblage of the apparatus for passing an electric discharge is shown diagramatically in the figure.

The detector used is a Ref. Gal. suitably shunted (Gambrell make) and actuated by a Germanium Crystal Diode 1N34 Sylvania. The ozoniser was excited under various applied fields of 50 cycles frequency and the discharge current in arbitrary units was observed when the ozoniser was in the dark (iD) and when irradiated externally from an incandescent bulb placed inside a chamber with pulley-operated shutters.

Observations.

Transformer Ratio=1 : 50 app.
Frequency of A.C. Supply=50 cycles/sec.
Source of irradiation=220 volts.
100 watts (glass) lamp
Detector=IN34 Germanium Crystal Diode
Resistance in the low tension wire=10,000 ohms.

Table No. 1

Applied potential in K.V.	iD	Light effect
·25	5	
·45	11	nil
·60	18	
——		→ glow starts in the discharge tube.
·75	24	
·9	↓	
·1	goes on increasing.	

Table No. 2 **Electric Discharge passed continuously for 4 hours.**

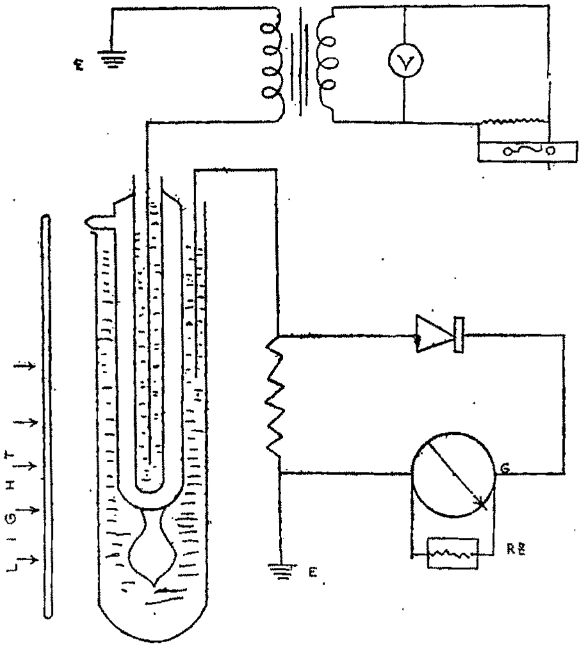
Applied potential in K.V.	iD	Light effect
·25	5	
·6	21	nil
·75	30	
·9		
	very large current	glow starts in the discharge tube.

Table No. 3 **Electric Discharge passed continuously for 8 hours.**

Applied potential in K.V.	iD	Light effect
·25	1	
·45	1	
·60	7	negligible.
·75	9	
·90	25	glow starts in the discharge tube.

The following important results emerge from the observations : —

1. Threshold potential Vm (where the vapour breaks down as a dielectric) initially is ·6 K. V. but if the discharge passes for a long time it abruptly rises to ·9 K. V. and remains constant at that voltage.



Diagrammatic representation of the general assemblage of apparatus used in passing electric discharge.

2. Inspection of the tube showed deposits of orange colour on the tube walls as "aging" proceeded.

3. Negligible Joshi Effect was observed.

4. C. R. O. studies showed complicated wave structure of the current with long thick but weak off-shoots in the positive and negative sides of the wave forms just near the peaks. These off-shoots were unaffected by light.

W. R. Grove (4) found that red phosphorus is produced when an electric discharge is sent through the vapours of ordinary phosphorus. These observations were verified by the authors as shown by the orange colour deposits on the walls.

Observations show that the threshold *potential* is low in the beginning and rises abruptly to a sufficiently high value as one variety changes into another. J. Boesken (5) considers that the transformation of yellow to red phosphorus is preceded by the decomposition of the P_4 molecules into P or P_2 mols., which at once condense to the red form. The reactions are symbolised :

$$P_4 \longrightarrow 2\,P_2 \quad \text{and} \quad n\,P_2 \longrightarrow P_2n$$

P. Jolibois (6) found that the velocity of transformation of yellow to red phosphorus increases with temperature. It is very slow even at 360° C. G. Lemoine (7) concluded that the transformation of one variety into another is reciprocal and balance reaction is involved. P-yellow$\rightleftharpoons$P-red. Therefore, the discharge of electricity through yellow phosphorus appears to be a very efficient and quick method of conversion of one veriety into another completely. Since after a few hours the threshold potential remains constant, indicating the complete conversion of one variety into another. This favours the views expressed by J. Boeska (vide supra).

The absence of Joshi Effect may be attributed to the following causes :—

1. Since the mols. are polyatomic before conversion and also so after, a negative effect may not be ordinarily observed.

2. Positive Joshi Effect is generally obtained when the pressure in the discharge tube is comparatively high (8,9) which is not the case with the ozoniser.

3. The nature of glass of the ozoniser plays a very important part (10) on the production of Joshi Effect because Joshi Effect is a "surface phenomenon" (11) dependent on the formation of absorbant-absorbate layer. Therefore, pyrex glass ozoniser show comparatively little effect as compared with hard Indian glass.

The complicated wave structure of the current is to be expected on general grounds. The non-suppression of off-shoots due to light indicates that the frequencies generated during the discharge are light-insensitive which are usually low frequencies.

My grateful thanks are to Prof. A. P. Saxena, for suggesting the problem, and to Dr. W. V. Bhagwat for the facilities offered.

REFERENCES

1. Joshi, 1943 Pres. Addr. *Proc. Ind. Sc. Cong.*; Chem. Sec.
2. Joshi. 1947, *Proc. Ind. Sc. Cong.* 1946, Abs. 26, Abs. 27 (Physics Sec).
3. Joshi. 1947, *Curr. Sci.*, **16** : 19.
4. Grove. W.R. 1863 J. *Chem. Soc.*, **16** : 263.
5. Boesken, J. *Chem weekl.*, 4, 200, 1907. *Proc. Acad. Amsterdam*, **9** : 613, 1907
6. Jolibois P. 1910. *Compt. Rend.* **149**. 287, 1909. **151** : 382.
7. Lemoine, G. *Bull. Soc. Chem.*, (2). **1** : 407, 1864. (2) **8** : 71, 1867.
8. Joshi & Cherian 1942. *Proc. Indian Sci. Cong.* Chem. Sec. Abs. 56.
9. Joshi & Raghavan. *Ibid.* 1942—65
10. Saxena, A.P. *Ibid.* 1952-Phy. Sec. Abs. 21.
11. Joshi 1945, *Curr. Sci.*, **14** : 35.

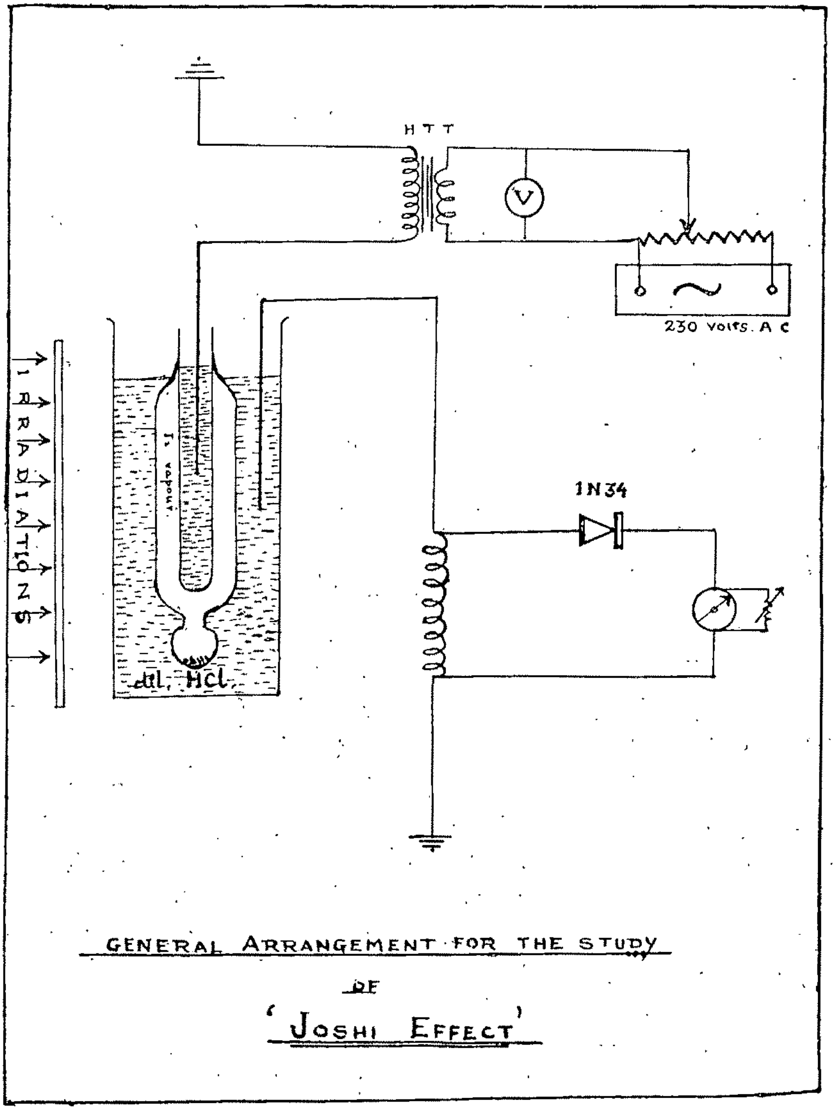
INCIDENCE OF JOSHI EFFECT IN THE HIGH FREQUENCY PART OF THE DISCHARGE CURRENT

By A. P. SAXENA, *Reader in Physics, Holkar College, Indore.*

Numerous experiments aided by C. R. O. studies (1, 2, 3, 4, 5, 6, 7) have clearly established the complicated structure of the current wave form containing numerous frequencies much higher than that of the exciting field. Exposure of the discharge tube to light usually, though not invariably, causes a current decrease (the negative Joshi Effect), which is found to be associated with the suppression of high frequencies. The effect is found to be maximum near/at Vm, the threshold potential, and decreases thereafter. It has been reported that the proportion of H.F. current in the discharge diminishes as the applied voltage has been increased (8, 9). The discharge current increases on increasing the voltage beyond Vm. Work in these laboratories (10), using very low resistance inductances in the low tension line, have shown a departure from the expected variation of discharge current with potential. Similar behaviour has recently been reported by Arnikar (11) under the heading "falling V-i characteristics." This anamolous behaviour of discharge current with potential may help in understanding the role of high frequencies in the production of Joshi Effect.

EXPERIMENTAL. The general assemblage of apparatus is shown diagramatically in the figure on p. 240. The figure represents the arrangement when detector used is a ref. galvanometer, suitably shunted (Gambrell make) and actuated by germanium crystal diode 1N34 Sylvania. The diode is reported to have a uniform rectification characteristic for various frequencies from 0 to 150 megacycles, so essential for the work of this type in hand.

The ozoniser used is a Siemen's type (glass) ozoniser containing powdered Iodine in contact with the solid phase. The ozoniser is in pretty long use and already sufficiently aged when the author started work.



Diagrammatic representation of the arrangement of the apparatus.

The ozoniser was excited under various applied fields of 50 cycles frequency and the discharge current was observed when the ozoniser was in the dark (iD) and when irradiated (iL) externally from an incandescent bulb placed inside a chamber with pulley operated shutters. From these data, the Net Joshi Effect $\triangle i = iD - iL$ and % Joshi Effect $= \frac{\triangle i}{iD} \times 100$ were calculated.

Observations :—

Transformer ratio = 1 : 50 app.
Frequency of A.C. Supply = 50 cycles/sec.
Source of irradiation = 220 volts. 100 watt (glass) bulb
Detector = 1N34 Germanium crystal diode.

Table No. 1 :—

Inductance in the low tension line=1 millihenry.
R= 5·5 Ohms

Exciting potential in K.V.	iD	iL	$\triangle i$	$\% \triangle i$
·35	102	0	102	100
·40	102	0	102	100
·45	102	0	102	100
·50	102	0	102	100
·55	102	0	102	100
·75	2	0	2	100

Table No. 2

Inductance in low tension line=·2 millihenry.
R=·9 Ohms

Exciting potential in K.V.	iD	iL	$\triangle i$	$\% \triangle i$
·35	12	0	12	100
·40	97	0	97	100
·45	97	0	97	100
·50	102	0	102	100
·60	102	0	102	100
·70	2	0	2	100
·75	2	0	2	100

DISCUSSIONS

The small inductances (H.F. Chokes) used in the circuit offer appreciable impedence only to very high frequencies, which choose a path of low impedence through the rectifier and get rectified. Hence the discharge current iD recorded in the tables represent the H.F. contribution to the total discharge current. By using higher values of inductances with negligible resistance, the range of rectified wireless spectrum can be broadened. The observation shows decrease in the discharge current with exciting potential indicating the decrease in the number and intensity of light sensitive frequencies (H F.). It is remarkable that the % Joshi Effect is nearly 100% current diminution under light at any potential. Therefore, it is clear that the number and intensity of light sensitive frequencies decrease with the exciting potential and at sufficiently high potential their contribution to the total discharge current is negligible. Hence it is to be expected that the % Joshi Effect should progressively decrease above Vm, being largest at/near Vm. The result is in close agreement to the findings of Joshi and others (8, 9), who explain the decrease in % Joshi Effect due to the decrease in the proportion of light sensitive frequencies in the discharge current with exciting potentials. The observations recorded above and aided by C.R.O. studies show that the reduced Joshi Effect at higher exciting potentials is due to decrease in the number and intensity of light sensitive frequencies (H.F.) with potential with simultaneous increase in the intensities (and probably the number) of light insensitive frequencies. (L.F.). Arnikar[11] arguing on Joshi Theory explains that the falling V-i characteristic would appear to be associated mainly with the H. F. component of the discharge Current. Potential variation of the wave form of the discharge current in dark and under light as seen in the C. R. O. fully corroborate this view.

REFERENCES

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1. Deb & Ghosh, 1948 *J. Indian chem. Soc.* **25** : 499.
2. Deb & Ghosh. 1946 *Sc. & Culture,* **12** : 17.
3. Joshi, 1943 *Banares Hindu Unir. J.* **8** : 99.
4. Joshi, 1944. *Nature,* **154** : 147.
5. Joshi, 1939. *Curr. Sci.,* **8** ; 548 ; 1945, **14** ; 175. 1946, **15**. 281, 1947. **16** 19.
6. Joshi, 1945. *Proc. Indian Acad. Sci.,* **22** ; 389.
7. Harris & Engel, 1951. *J. Chem. Phy.,* **19** : 514.
8. Warburg. 1924. *Tech. Physic.,* **165**.
9. Prasad & Jain, 1947. *Proc. Indian Acad. Sci.,* **25** : 515.
10. Saxena, A. P. 1951. *J. I. C, S.* **28** : 239.
11. Arniker, 1952. *Proc. Indian Sci. Cong. Phy. Sec. Abst.* 17.

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CONTENTS

PAGE

1. Ourselves i
2. Foreword iii
3. Messages iv
4. Correlation of the Gondwanas based on the evidence of Plant Fossils By SHIVDAYAL SAXENA 1
5. Floral Anatomy in relation to taxonomy. By V. PURI ... 15
6. Contributions to our knowledge of the structure of the Placenta of *Panthera tigris tigris* (Linn.). By SHYAMA CHARAN SRIVASTAVA 37
7. Some Gall-midges (Itonididae:Diptera) associated with *Mangifera indica* Linn. in India and their Galls. By M.S. MANI 47
8. Four New species of Chalcidoidea (Parasitic Hymenoptera) from India. By CHANDY KURIAN 55
9. Descriptions of Four New and Record of one known Bethyloidea (Parasitic Hymenoptera) from India. By CHANDY KURIAN 63
10. Contributions to our knowledge of Fig-insects (Chalcidoidea: Parasitic Hymenoptera) from India. II.—On a New species of *Lipothymus* Grandi from *Ficus hispida* Linn. By K.J. JOSEPH 73
11. On Some Arthropod microfossils from India. By V.P. GEORGE 83
12. Biosynthesis of Vitanins. By H.N. DE. 109
13. The Effect of Nitrogen Fertilization on the chemical composition of the Leaves of Peach trees. By V.S. CHHONKAR. ... 131
14. Predictions of the Ionospheric Conditions from the Observations of Fading Pattern of Radio-signals. By RAM NETI SINGH... 143
15. Condensation of Aldehydes with Phenylacetic acid. Part I.—Condensation of benzaldehyde, salicylaldehyde and anisaldehyde with phenylacetic acid. By R.N. SINGH *et al.* ... 153
16. Muta-Rotation of d-oxymethylene Camphor and its Condensation products with primary amines and of d-oxymethylene carvone. By S.G. HARMALKAR 161
17. Hydrogen Bonding and Resonance from the study of Parachor. By W. V. BHAGWAT *et al.* 169
18 Recent Advances in our knowledge of Fluorescent Reagents. By C. N. HAKSAR 177

19. Action of Drugs and Ions on unstriated muscle. By SUNITA INDERJIT SINGH *et al.* 186

20. A Clinical Study of three hundred and eighty-three cases of typhoid fever at the S. N. Hospital, Agra. By K.N. GOUR 189

21. Studies of the Role of Fungi in Pulmonary disease. By P.N. WAHI. 205

22. Crohn's Disease of the Ileocaecal region. By S.P. SRIVASTAVA 225

23. Allotropic Modification of a substance and the Joshi Effect. ... By PENDSE. 233

24. Incidence of Joshi Effect in the High Frequency part of the Discharge current. By A.P. SAXENA. 239

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